The Effects of Acute Stress on Behavioural and ERP Measures of Human Attentional Networks

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Statement of Sources

I declare that this report is my own original work and that contributions of others have been duly acknowledged.

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Caleb Stone

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Abstract

Neurobiological models suggest that acute stress facilitates bottom-up stimulus processing while impairing top-down executive control. To test this hypothesis, the present study investigated the effects of acute stress on behavioural (reaction time and accuracy) and electrophysiological (N1 and P3 ERP component amplitude) measures of the alerting, orienting, and executive attentional networks. Forty-three right-handed females aged 18-34 were recruited and performed the Attention Network Test (ANT) before and after the Maastricht Acute Stress Test (MAST) or a non-stressful MAST-placebo. Subjective Units of Distress Scale (SUDS) ratings and salivary cortisol concentrations were further collected as manipulation checks. While the manipulation checks revealed a successful stress induction, the hypothesised detrimental effect of acute stress on executive function was not found for reaction time (p=.524), accuracy (p=.657), or P3 amplitude (p=.408). Similarly, the hypothesised beneficial effect of stress on bottom-up stimulus processing was not found for measures of reaction time (p=.857) or N1 amplitude (p=.107). Supplementary analyses indicated that these findings were unrelated to the magnitude or duration of the stress response induced by the MAST, instead suggesting that the ANT itself may be insensitive to the effects of acute stress. Future research may utilise alternate versions of the ANT to address this possibility.

The acute stress response represents an evolutionarily conserved mechanism of adaption to real or perceived threats to homeostasis or wellbeing (Herman et al., 2016). While ultimately serving to increase the chance of survival, activation of the stress response has been shown to differentially affect aspects of cognitive functioning (Shields, Sazma, & Yonelinas, 2016). Specifically, finite cognitive resources are preferentially allocated to cognitive functions that will confer the greatest survival advantage, such as those involved in threat detection (e.g., sensory processing) and guidance of future behaviours (e.g., memory formation). At the same time, higher-order cognitive functions are impaired (e.g., mental flexibility) (Vogel, Fernandez, Joels, & Schwabe, 2016). These shifts in cognitive functioning arise from stress-induced changes in the brain's neurochemistry, and in the functional connectivity between brain regions such as the prefrontal cortex and the amygdala (Arnsten, 2009, 2015; Vogel et al., 2016).

One aspect of cognition crucial for ensuring survival in stressful or threatening situations is attention. Attention refers to the ability to select physical or mental stimuli for conscious perceptual processing (Raz, 2004). According to Arnsten (2009), stress switches attentional regulation from top-down goal-directed processing to bottom-up stimulus-driven processing, thereby facilitating the detection of salient threat-related cues in the environment. In the current study, we aimed to capture the effects of acute stress on attention by examining both behavioural and electrophysiological measures of performance during the Attention Network Test (ANT) (Fan, McCandliss, Sommer, Raz, & Posner, 2002). The ANT was developed within the framework of the influential attentional network theory (Posner & Petersen, 1990), and provides a measure of both bottom-up and top-down components of attention. Despite its usefulness, however, to our knowledge no

previous research has utilised the ANT in the study of stress-induced alterations in attention.

Attentional Network Theory

According to Posner and Petersen (1990), the human attentional system consists of three interrelated attentional networks responsible for alerting, orienting, and executive functions, respectively. Neuroimaging (Fan, McCandliss, Fossella, Flombaum, & Posner, 2005) and behavioural (Fan et al., 2002) research has revealed a degree of anatomical and functional independence between these networks, while pharmacological studies suggest that each network is primarily mediated by a distinct neurotransmitter system (Petersen & Posner, 2012). As such, attentional network theory provides a plausible framework within which to examine the differential effects of stress on attention.

The *alerting network* is responsible for an individual's level of vigilance and arousal (Fan & Posner, 2004; Posner & Petersen, 1990). This network primarily relies on noradrenergic signalling from the locus coeruleus of the brainstem to thalamic, parietal and frontal brain regions (Fan et al., 2005; Petersen & Posner, 2012). Activation of the alerting network is suggested to reduce the time required for response selection by increasing vigilance towards a stimulus, therefore improving reaction times to target stimuli (Fan & Posner, 2004; Petersen & Posner, 2012; Posner, 2008).

The *orienting network* is involved in selective attention to sensory stimuli, and is associated with regions of the parietal cortex, thalamus, frontal eye fields, and the superior colliculus (Callejas, Lupianez, Funes, & Tudela, 2005; Fan et al., 2005; Fan & Posner, 2004; Posner, 2008). The orienting network is primarily mediated by cholinergic signalling arising from the basal forebrain (Petersen & Posner, 2012).

Like the alerting network, activation of the orienting network reduces response times to sensory stimuli (Posner & Petersen, 1990).

Finally, the *executive network* primarily involves dopaminergic signalling in the anterior cingulate cortex and areas of the prefrontal cortex (Fan & Posner, 2004). The executive network is implicated in conflict monitoring/resolution and the inhibition of irrelevant information (Fan & Posner, 2004; Petersen & Posner, 2012), and as such is involved in tasks requiring top-down attentional regulation, such as Stroop and flanker style tasks (Fan et al., 2002; Wang & Fan, 2007).

The Attention Network Test (ANT)

To assess the independent functioning of each network, Fan, McCandliss, Sommer, Raz, and Posner (2002) developed the ANT. The ANT requires participants to indicate the direction of a target stimulus (left or right facing arrow) appearing above or below a fixation cross following one of four equiprobable cue conditions (i.e. no cue, central cue, double cue, spatial cue). Results indicate that reaction times are faster when a central cue (at fixation) or a double cue (above *and* below fixation) is presented relative to no cue, and when a spatial cue (above *or* below fixation; 100% valid) is presented relative to the central/double cue. Additionally, each target stimulus is flanked by either congruent (same direction), incongruent (opposite direction), or neutral (straight lines) distractor arrows. Reaction times are slower and accuracy is poorer for incongruent trials relative to congruent trials, with negligible differences between congruent and neutral conditions (Fan et al., 2002).

To compute the efficacy of each network, three reaction time scores are calculated from the ANT (Fan et al., 2002). The *alerting effect* is derived by subtracting the mean reaction time for the double or central cue condition from the mean reaction time of the no cue condition, yielding an approximately 50ms

improvement. The *orienting effect* is calculated by subtracting the mean reaction time of the spatial cue condition from the mean of the central cue condition, again yielding an approximately 50ms improvement. Finally, the *executive control effect* is calculated by subtracting the mean reaction time of the congruent trials from that of the incongruent trails, yielding an approximately 80ms improvement (Fan et al., 2002).

These improvements in performance likely relate to changes in the neurophysiology underpinning each network. In relation to the alerting network, central/double cues provide information about *when* a target stimulus will occur, leading to increased vigilance mediated by the release of noradrenaline. Noradrenaline exerts a neuromodulatory influence on target neurons by differentially affecting their firing rates (Aston-Jones & Cohen, 2005a). Specifically, noradrenaline increases evoked neural firing (i.e. in response to input) while decreasing spontaneous neural firing (Aston-Jones & Cohen, 2005a; Sara, 2009). This effect has been described as an increase in neural responsivity, or *gain*, such that the influence of excitatory and inhibitory inputs is exaggerated (e.g., from sensory stimuli) (Aston-Jones & Cohen, 2005b; Nieuwenhuis, Aston-Jones, & Cohen, 2005).

Acetylcholine similarly performs a neuromodulatory role in the cortex (Picciotto, Higley, & Mineur, 2012), which may account for the reduction in reaction times following the presentation of spatial cues during the ANT. Spatial cues provide information about both when and *where* a target stimulus will occur, therefore promoting attentional orienting. Parietal brain regions and the superior colliculus are thought to be involved in the disengagement and reorientation of attention, respectively, while the thalamus improves processing of stimuli at the newly attended location (Callejas et al., 2005). According to Picciotto et al. (2012),

acetylcholine enhances signals coming from the thalamus, thereby increasing the sensitivity of cortical neurons to incoming stimuli. As such, the presentation of spatial cues leads to the selective deployment of attention and subsequently improves sensory processing at target locations.

Finally, the anterior cingulate cortex is suggested to be heavily involved in identifying response conflict, such as during incongruent flanker trials when both task-relevant and irrelevant stimuli are presented simultaneously and are associated with opposing responses (van Veen, Cohen, Botvinick, Stenger, & Carter, 2001). Following identification of a response conflict, the anterior cingulate sends projections to the dorsolateral prefrontal cortex, which may subsequently engage in top-down response control (Carter & van Veen, 2007). Engagement of additional brain areas during conflict monitoring may therefore lead to the increased reaction time for incongruent compared to congruent trials (van Veen et al., 2001).

Electrophysiological Markers of Attentional Networks

In addition to behavioural paradigms, electroencephalography (EEG) provides a valuable resource in the study of attention by enabling detailed analysis of the time course of information processing on a scale of milliseconds to seconds (Luck, Woodman, & Vogel, 2000; Woodman, 2010). In the study of attention, raw EEG data is often converted into electrical waveforms known as event-related potentials (ERPs). ERPs provide a measure of the averaged electrical activity of the brain, time-locked to the onset of a particular stimulus or response (Woodman, 2010). Discrete components exist within the ERP waveform, defined by their polarity (positive, P, or negative, N) and their timing (temporal order or latency). For example, the N1 component is the first negative peak in the ERP waveform, appearing at approximately 150-200ms post-stimulus (Luck, 2014). ERP components

index specific cognitive processes, including early selective attention or higher-order perceptual mechanisms such as error processing (Woodman, 2010). As such, ERP data allows for neurophysiological correlates of task performance to be compared between conditions or groups.

In relation to the ANT, Neuhaus et al. (2010) authored a seminal paper on the ERPs associated with each attentional network. Neuhaus et al. (2010) replicated the expected pattern of reaction time results described by Fan et al. (2002), coupled with amplitude modulations of the N1 and P3 ERP components. Specifically, N1 amplitude was incrementally augmented at occipital sites by the presentation of the double and spatial cues relative to the no cue and central cue conditions, respectively (i.e. spatial>central/double>no cue). Furthermore, P3 amplitude was increased for incongruent relative to congruent stimuli in frontocentral brain regions and decreased at centroparietal brain regions. Similar results have since been observed in additional studies (e.g., Galvao-Carmona et al., 2014; Williams et al., 2016).

According to Neuhaus et al. (2010), these findings reflect greater engagement of sensory processing (N1) and response inhibition (P3) mechanisms during the relevant ANT trials. Specifically, the N1 component has long been associated with selective attention (Hillyard, Hink, Schwent, & Picton, 1973). Numerous studies have demonstrated augmented N1 amplitudes for attended versus unattended stimuli, including stimuli appearing at validly cued timepoints and spatial locations (e.g., Hopf & Mangun, 2000; Lange & Roder, 2006). These results are thought to reflect enhanced sensory gain control as described above, with attention amplifying neural responses to attended stimuli (Hillyard, Vogel, & Luck, 1998). As such, incremental increases in N1 amplitude reflect the neurophysiological correlates of incremental

decreases in reaction times following activation of the alerting and orienting networks (Neuhaus et al., 2010).

The P3 component, on the other hand, appears at a latency of 300-600ms post-stimulus and reflects higher-order cognitive control processes used to inform decision making and response selection (Nieuwenhuis et al., 2005). In go/no-go and flanker style tasks requiring response inhibition and conflict monitoring, the amplitude of the P3 is increased anteriorly during no-go or incongruent flanker trials relative to go or congruent trials, respectively (Groom & Cragg, 2015; Jonkman, Lansbergen, & Stauder, 2003). According to Neuhaus et al. (2010), this frontocentral amplitude modulation stems from the activity of the anterior cingulate cortex and executive control network to inhibit irrelevant information and responses. This conclusion supports findings that the anterior cingulate cortex is likely the major generator of the anterior P3 component (Schmajuk, Liotti, Busse, & Woldorff, 2006), and receives extensive dopaminergic innervation (Allman, Hakeem, Erwin, Nimchinsky, & Hof, 2001).

Stress and the Attentional Networks

The neurotransmitters and brain regions associated with the attentional networks overlap considerably with those of the acute stress response, providing a plausible neurobiological mechanism by which stress may alter attentional processes. As mentioned above, current models propose that stress induces changes in the brain's neurochemistry and functional connectivity, initiating a shift from complex cognitive functions controlled by the prefrontal cortex to habitual responses driven by primitive brain circuits involving the amygdala (Arnsten, 2009, 2015; Vogel et al., 2016). In particular, stress-induced increases in catecholamines, such as dopamine and noradrenaline, are suggested to take the prefrontal cortex "off-line"

while strengthening the primary sensory cortices, ultimately biasing cognition to enhance processing of information related to the stressor (Arnsten, 2009; Shields et al., 2016).

While the complete biochemical stress response involves a myriad of signalling pathways and sub-responses, two primary brain regions are responsible for eliciting and maintaining its major components. Following exposure to a stressor, sensory information is initially sent to brainstem nuclei such as the locus coeruleus, known to be among the most stress sensitive structures in the entire brain (Sanger, Bechtold, Schoofs, Blaszkewicz, & Wascher, 2014; Ulrich-Lai & Herman, 2009). The release of noradrenaline from the locus coeruleus is subsequently increased. As detailed above, the activity of the locus coeruleus is intrinsically linked to the functioning of the alerting network, with noradrenaline acting to increase sensory gain. Acute stress will therefore likely influence performance on behavioural paradigms such as the ANT by increasing vigilance and reducing reaction times to stimuli (Aston-Jones, Rajkowski, Kubiak, & Alexinsky, 1994; Hermans et al., 2011).

The locus coeruleus shares stimulating connections with the hypothalamus, the second major brain region involved in eliciting the stress response (Tsigos & Chrousos, 2002). Once activated, hypothalamic nuclei trigger an immediate response from the sympathetic nervous system, known as the “fight-or-flight” response, to prepare the body for the stressor (Everly & Lasting, 2013). The hypothalamus is also responsible for triggering a delayed hormonal response to stress via activation of the hypothalamic-pituitary-adrenal (HPA) axis, which stimulates the secretion of the stress hormone cortisol from the adrenal glands (Herman et al., 2016; Tsigos & Chrousos, 2002).

Once secreted, cortisol readily crosses the blood-brain barrier and binds glucocorticoid and mineralocorticoid receptors distributed diffusely throughout the brain. In particular, the glucocorticoid cortisol receptor is expressed extensively in the prefrontal cortex and experiences substantially increased occupation rates during acute stress (Lupien, Maheu, Tu, Fiocco, & Schramek, 2007). This elevated glucocorticoid receptor occupation is suggested to be partially responsible for the effects of stress on cognition (Lupien et al., 2002). However, as the glucocorticoid and mineralocorticoid receptors exist intracellularly, they exert their influence by altering gene transcription and expression over a time scale of minutes to hours (Gagnon & Wagner, 2016). More recently, a second, membrane-bound mineralocorticoid receptor has been identified, possessing the ability to rapidly alter cellular activity via non-genomic pathways (Vogel et al., 2016). It is suggested that the action of this nongenomic receptor subtype shifts cognitive resources away from high-order functions to less cognitively demanding processing, allowing for rapid responding to potentially threatening stimuli under stress (Joels, Sarabdjitsingh, & Karst, 2012; Vogel et al., 2016). While cortisol is not directly linked to the attentional networks, the actions of both the membrane-bound and intracellular cortisol receptors provide a plausible mechanism by which acute stress may impair performance on measures of executive function, such as during the incongruent flanker trials of the ANT.

Finally, acute stress has been shown to stimulate the release of both dopamine and acetylcholine (Imperato, Puglisi-Allegra, Casolini, & Angelucci, 1991; Wand et al., 2007). An inverted “U” shaped dose-response curve is said to exist for dopamine, such that increased or decreased concentrations impair cognitive performance (Matuszewich, McFadden, Friedman, & Frye, 2014). This finding is consistent with

the suggestion that stress-induced increases in catecholamines take the prefrontal cortex "off-line" (Arnsten, 2015), and that attention is disrupted in disorders such as schizophrenia and Parkinson's disease (Nieoullon, 2002). Given the reliance of the executive attention network on dopaminergic signalling, these findings provide a second mechanism by which acute stress may impair performance on incongruent flanker trials of the ANT. For acetylcholine, results from animal studies suggest improved performance on attentional tasks associated with acetylcholine release and receptor stimulation (Howe et al., 2010; Parikh, Kozak, Martinez, & Sarter, 2007). Moreover, as described above, acetylcholine acts to increase the sensitivity of cortical neurons to incoming sensory signals from the thalamus (Picciotto et al., 2012). As such, increased acetylcholine release driven by acute stress may facilitate performance on measures of the orienting network, which is known to involve cholinergic neurotransmission (Petersen & Posner, 2012).

ERP Studies of Acute Stress

Despite the usefulness of EEG for studying cognition, comparatively little research has looked specifically at the effects of acute stress on its ERP correlates. Nevertheless, several ERP studies have demonstrated a dissociable effect of stress on the neural correlates of early sensory and late perceptual processing during a variety of attentional tasks, supporting the neurophysiological overlap between the attentional system and acute stress response. Indeed, amplitude modulations of both the N1 and P3 ERP components are consistent findings from studies looking specifically at the effects of acute stress on attention (Jiang et al., 2017; Qi, Gao, & Liu, 2018; Sanger et al., 2014; Shackman, Maxwell, McMenamin, Greischar, & Davidson, 2011) and other aspects of cognition (Dierolf, Fechtner, Bohnke, Wolf, & Naumann, 2017; Jiang & Rau, 2017).

For example, using an electric shock stress induction method, Shackman, Maxwell, McMenamin, Greischar, and Davidson (2011) found increased N1 amplitude over frontocentral and occipital electrode sites, and decreased P3 amplitude over centroparietal regions for threat trials relative to control trials in a flanker-style speeded response task. Shackman et al. (2011) suggested that these findings reflected an attentional bias stemming from modulation of the visual cortex by inputs from the amygdala, and a diversion of attentional resources away from top-down control to facilitate bottom-up processing. Collectively, these results support the notion of a stress-induced switch from a "controlled task-directed mode" to a "vigilant threat assessment mode" of cognition (Shackman et al., 2011, p. 1160).

Similarly, amplitude modulations of both the N1 and P3 were observed in a recent study by Qi, Gao, and Liu (2018). To induce stress, participants performed a mental arithmetic task under time pressure and evaluative threat, followed by a stimulus discrimination task in which participants indicated the direction of a target arrow. A significant main effect of condition for P3 amplitude at midline electrodes was found, such that the P3 was reduced following the stress relative to the control condition. Qi et al. (2018) also identified increased amplitude of the N1 at midline electrode sites and faster reaction times following stress. These results support those of Shackman et al. (2011) in identifying enhanced neural indices of early sensory processing and a reduction of cognitive resources directed towards deeper levels of stimulus processing (Qi et al., 2018).

Finally, looking specifically at response inhibition, Dierolf, Fetchtner, Bohnke, Wolf, and Naumann (2017) recruited participants to perform a go/nogo task before and after the socially evaluated cold pressor test (SECPT); a common stress induction method involving hand immersion in ice water and social evaluation by an

experimenter (Schwabe, Haddad, & Schachinger, 2008). Measures of salivary cortisol were also taken throughout the experiment. While there was no difference in behavioural performance, participants classified as high cortisol responders recorded a reduced no-go P3 amplitude at frontocentral electrodes post- relative to pre-stress.

Current Study Aims and Hypotheses

While the studies outlined above provide empirical support for a differential effect of acute stress on the neural correlates of attentional networks, they do so indirectly and without capturing the relevant behavioural performance indices offered by the ANT. Moreover, discrepancies exist between the specific brain regions/electrode sites examined and those Neuhaus et al. (2010) identified as being associated with the ANT. As such, the current study aimed to specifically address the influence of acute stress exposure on the relevant behavioural and electrophysiological measures of human attentional networks. While the original ANT comprised four cue and three flanker congruency conditions, the double cue and neutral flanker conditions were removed for the current study. This adapted version of the ANT allowed for adequate trial numbers for each cue by flanker congruency condition while still retaining a feasible task length, and has been utilised in other studies (e.g., Fan et al., 2005).

It was hypothesised that if acute stress facilitates bottom-up attentional processing, it may therefore improve measures of the orienting and alerting networks such that following a stress manipulation stressed participants would display (a) faster reaction times for central and spatial cues compared to controls (i.e. greater alerting and possibly orienting effects), and (b) greater N1 amplitude for central and spatial cues relative to controls. Note that because the orienting effect is a difference score involving reaction time for central cues, its magnitude may not change.

Contrarily, if acute stress reduces top-down attentional control, it may therefore impair measures of the executive network such that stressed participants would display (c) slower reaction times and/or poorer accuracy for incongruent stimuli compared to controls (i.e. greater executive control effect), and (d) a smaller congruency effect for P3 amplitude compared to controls.

Method

Participants

A priori power calculations (G-Power 3.1.9.2) indicated that a sample size of 36 would allow for detection of a small-moderate effect (f=0.20, β=.20, α=.05). Forty-three right-handed females aged 18-34 years (M_{age}=22.15, SD=4.45) were recruited, with three participants excluded due to poor accuracy (<70%), three excluded due to technical issues with EEG recording equipment, and a final participant excluded from analyses involving cortisol for being an extreme outlier. A final sample of 36 (18 control) was therefore included for all analyses involving cortisol, and 37 (19 control) for all remaining analyses. Participants were reimbursed with a $30 gift voucher or three hours course credit. Participants were recruited via the University of Tasmania's online research participation system, flyers, and worth-of-mouth.

Due to known sex differences in stress response activation (Zimmer, Basler, Vedder, & Lautenbacher, 2003), only female participants were recruited to prevent possible confounds. Exclusion criteria included current psychiatric diagnoses and/or use of psychiatric medication, serious physical or neurological illnesses, high levels of psychological distress (as indicated by scores >30 on the Kessler Psychological Distress scale (K10); Kessler et al., 2002), illicit drug use in the previous six months, current tobacco use, problematic alcohol use (as indicated by scores >16 on the

Alcohol Use Disorders Identification Test (AUDIT); Saunders, Aasland, Babor, de la Fuente, & Grant, 1993), current pregnancy, sensitive skin, uncorrected problems with hearing or vision, previous experience with the Maastricht Acute Stress Test (MAST) (Smeets et al., 2012), left-handedness, and first languages other than English.

Materials

Screening questionnaire. Participants' basic medical history, demographic information, and drug use history were obtained prior to their participation via an online screening questionnaire (see Appendix A). The questionnaire also included the K10 (Kessler et al., 2002) and AUDIT (Saunders et al., 1993) to screen for those with high psychological distress and heavy alcohol use/alcohol dependence, respectively.

The K10 (Kessler et al., 2002) comprises 10 items assessing respondents' experience of negative emotions over the previous four weeks. Items are rated on a five-point Likert scale ranging from 1 = 'none of the time' to 5 = 'all of the time'. Possible scores range from 10 to 50, with scores of >30 demonstrating perfect specificity in identifying people who meet criteria for current anxiety or affective disorders (Andrews & Slade, 2001). Reliability and validity of the K10 have been established among a range of populations (Kessler et al., 2002; Slade, Grove, & Burgess, 2011).

The AUDIT (Saunders et al., 1993) is a 10 item self-report measure of alcohol consumption. Items assess the domains of hazardous alcohol use (related to frequency and dosage), harmful alcohol use (related to negative consequences), and dependence symptoms, and are scored on a variety of scales ranging from 0 to 4. Total scores of ≥16 indicate problematic alcohol use (Babor, Higgins-Biddle,

Saunders, & Monteiro, 2001). Reliability, internal consistency, and sensitivity to detect problematic alcohol use are high (Allen, Litten, Fertig, & Babor, 1997; Reinert & Allen, 2007).

Attention Network Test (ANT). An adapted version of the ANT (Fan et al., 2002) was presented using Neuroscan Stim^2 software on a desktop computer (see Figure 1). Stimuli consisted of five horizontal arrows appearing either above or below a fixation cross centred on the screen. Each arrow was 0.4cm high by 0.5cm wide (total stimulus width: 2.8cm, visual angle: 2.67˚) and appeared 1cm above or below fixation (visual angle: 0.96˚). All stimuli were white presented on a black background.

During the ANT participants were required to indicate the direction of the middle target arrow using either the index (left-facing arrow) or middle finger (right-facing arrow) of their right hand on a response pad. As the MAST requires immersion of the non-dominant hand in ice water (i.e. participants' left hands), only right-handed responding was used throughout the study. Participants were instructed to ignore the two flanker arrows present on either side of the target arrow. Flanker congruency, stimuli location, and target arrow direction all occurred with equal probability.

The ANT consisted of 10 practice trials, followed by 480 experimental trials broken into four blocks, each separated by a rest period. There were 80 trials per cue and flanker congruency conditions, exceeding that commonly seen in other ERP studies using the ANT (e.g., Galvao-Carmona et al., 2014; Neuhaus et al., 2010; Williams et al., 2016) to improve the ERP signal-to-noise ratio. Each trial began with 400ms of fixation, followed by 100ms of cue presentation (either no cue, central cue, or spatial cue; pseudo-randomised and equiprobable). The central and spatial cue

consisted of an asterisk (0.4cm high) which appeared at either fixation (central cue) or above/below fixation at the location of the upcoming stimulus (spatial cue; visual angle: 0.96˚). Following cue presentation, fixation occurred for another 400ms. Finally, stimulus presentation occurred for 1000ms. Four randomised inter-trial intervals of 1000ms, 1100ms, 1200ms, and 1300ms occurred with equal probability to reduce latency jitter of ERPs. The total task length of the ANT was approximately 25 minutes.

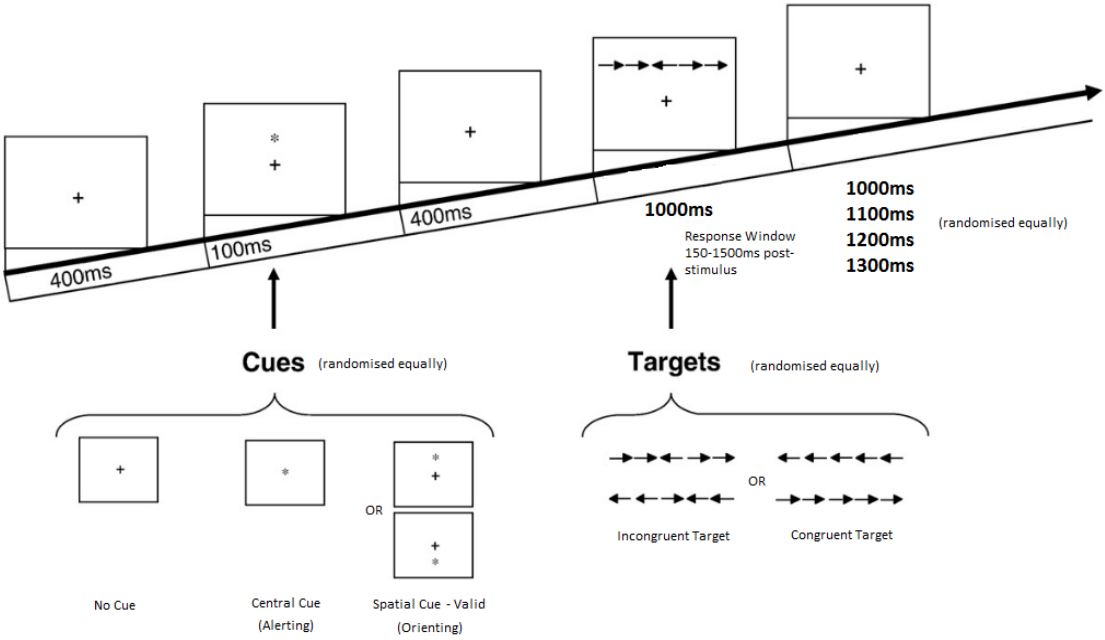


Figure 1. Schematic of the Attention Network Test, adapted from Neuhaus et al. (2010).

Maastricht Acute Stress Test (MAST). Participants were randomly allocated to complete either the MAST or a non-stressful MAST-placebo and were blind to their allocation until the stress manipulation occurred (Smeets et al., 2012). Both tasks were presented on a desktop computer and involved a five-minute instructional period, followed by 10 minutes of experimental trials. In the MAST

condition, this involved alternating trials of physiological and psychological stress. Physiological stress was induced by immersion of the left hand in ice water (0-2˚C), while psychological stress was induced by a challenging mental arithmetic task (counting backwards from 2043 in 17s out loud). During the mental arithmetic task, the experimenter(s) provided negative feedback for inaccuracy and speed.

To further increase psychological stress, participants were led to believe that they were being filmed. To simulate filming, a webcam was set facing participants and connected to a computer facing the experimenter. Participants were further led to believe that the length of each trial would be randomly chosen by the computer. In actuality, trial lengths were set (see Figure 2) and no filming occurred. The use of deception in the MAST is justified as a means of increasing uncertainty and psychological stress, with minimal risk of lasting distress (Smeets et al., 2012). The MAST has been shown to elicit strong stress responses from both the neuroendocrine and sympathetic nervous system, equalling or exceeding other common stress induction methods (Shilton, Laycock, & Crewther, 2017; Smeets et al., 2012).

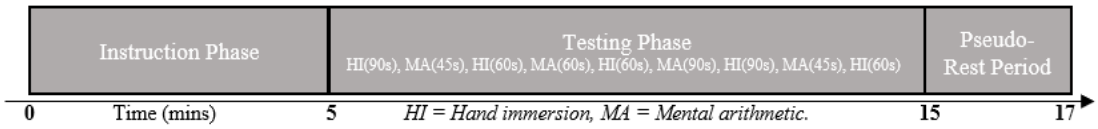


Figure 2. Maastricht acute stress test procedure order (Smeets et al., 2012)

Participants in the MAST-placebo condition completed 10 minutes of interchangeable trials of hand immersion in lukewarm water and a simple counting task (counting from 1 to 25 out loud). The counting task was completed without evaluation, and participants were not deceived about filming. Participants in both the MAST and MAST placebo conditions, however, were misled about the total length

of the procedure and were instructed that a “rest period” would occur in which measurements of stress response activation were to be collected (detailed below). In actuality, the rest period was the end of the task and was labelled as a rest period to prevent relief onset from affecting stress response measures (Smeets et al., 2012)

Subjective Units of Distress Scale (SUDS). As a manipulation check, participants completed the SUDS (see Appendix B; Wolpe, 1969) at baseline, 5-, 30-, and 45-minutes following the completion of the MAST/MAST-placebo. The SUDS is a single item questionnaire requiring participants to indicate their current level of distress on a “distress thermometer”, with possible scores ranging from 0 = ‘totally relaxed’ to 100 = ‘highest distress ever felt’. The SUDS has been shown to possess good psychometric qualities (Kim, Bae, & Park, 2008).

Saliva samples. In addition to completing the SUDS, participants provided a saliva sample (1mL per sample) at the same four-time points, allowing for biochemical analysis of the stress response. Saliva samples were collected using the passive drool method and stored at -20˚Celsius, before being assayed for levels of cortisol, sex hormones, and endocannabinoids using mass spectrometry (data for sex hormones/endocannabinoids not included). Known peak concentration times for these compounds therefore dictated the timing of saliva collection/SUDS completion, with salivary cortisol peaking 20 to 40 minutes following stress exposure (Gagnon & Wagner, 2016).

Electroencephalographic (EEG) recording. EEG data were collected using Neuroscan 4.5 software and a 32 channel Quik-Cap with Ag/AgCl sintered electrodes placed according to the international 10-20 system (Jasper, 1958) and referenced to linked mastoids. Horizontal and vertical electrooculographic activity was gathered from the outer canthi and from above and below the left eye,

respectively. Electrical impedance was kept below 5kΩ and data were sampled continuously at a rate of 1000Hz.

Data editing involved merging of behavioural and continuous EEG data, followed by a Zero-phase-shift low pass filter (30Hz, 24dB/Oct). An ocular artefact reduction procedure was employed, via regression computation and artefact averaging, to reduce the effects of eye blinks on data from other electrodes. Epochs of 1000ms starting 100ms before stimulus onset were then extracted, followed by baseline correction and rejection of trials containing artefacts of ± 70μV. N1 component amplitudes were gathered from the Oz electrode to capture attentional modulation occurring in the visual cortex and defined as the maximum peak between 120 and 190ms. P3 component amplitudes were gathered from the Fz electrode to capture the congruency P3 effect and defined as the maximum peak between 300 and 600ms. Time frames were based and inspection of grand averaged waveforms, with automated peak detection followed by manual inspection and correction.

Procedure

This research was approved by the University of Tasmania Human Research Ethics Committee (see Appendix C). Following completion of the screening questionnaire, eligible participants were invited to attend a 3-hour experimental session at the University campus. All participants were scheduled between 11:00am and 6:00pm to account for natural circadian rhythms in cortisol release (Joels et al., 2012). Upon arrival, participants were provided with an information sheet (see Appendix D) and gave informed consent (see Appendix E). Participants then completed a questionnaire assessing the recent use of drugs, alcohol, or medications to ensure eligibility (see Appendix F). Following this, an optional hair sample was collected for a separate study (data not included).

Following EEG set-up participants were seated in an electrically shielded room approximately 60cm away from a computer screen. The ANT and a nBack task were then completed, providing a baseline measure of attention and working memory (data for nBack not included). Participants were instructed to complete the tasks as fast and as accurately as possible. The presentation order of the two tasks was fixed, such that the ANT always preceded the nBack. This was to ensure that saliva samples could be taken at the appropriate time points. Following the baseline tasks, participants provided their first saliva sample/SUDS rating.

Next, the MAST/MAST placebo was administered as described above. In the "rest period" of the MAST/MAST placebo, participants provided their second saliva sample/SUDS rating. EEG electrical impedances were then rechecked. The second ANT was then completed, followed by the third saliva sample/SUDS rating. Finally, participants completed the second nBack task and fourth saliva sample/SUDS rating. Following removal of EEG equipment, participants were debriefed about all uses of deception and thanked for their participation.

Design and Data Analysis

A mixed experimental design was used for this study. Behavioural dependent variables were reaction time (ms) and accuracy (% correct responses), while electrophysiological dependent variables were peak amplitude of the N1 and P3 ERP components (μV). Before the data were collated, trials with reaction times less than 150ms or more than three standard deviations above individual means were excluded. Data were analysed using IBM SPSS Version 23. Demographic variables were analysed with independent-samples *t*-tests, while SUDS scores/salivary cortisol measurements were analysed using two 2(Condition: control, stress) x 4(Time: Baseline, 5, 30, 40) mixed ANOVAs. One-sample *t*-tests were conducted to

determine discrepancies between the mean recorded time of saliva sample measurements and their respective target times.

To address hypotheses relating to behavioural and electrophysiological dependent variables, four 2(Condition: control, stress) x 3(Cue: no, central, spatial) x 2(Flanker Congruency: congruent, incongruent) x 2(Time: pre, post) mixed ANOVAs were run. Where more than two levels of within-groups variables were present, Greenhouse-Geisser corrections were applied to correct for likely violations of sphericity. Interactions and main effects of theoretical relevance were followed up with analysis of simple effects and Bonferroni adjusted pairwise comparisons. Where violations of the assumption of homogeneity of variance occurred (as indicated by significant Levene's tests), non-parametric Mann-Whitney U tests were performed.

Partial eta squared (η_p^2) values are presented for omnibus ANOVAs as a measure of effect size, while Hedge's *g* is presented for pairwise comparisons. Partial eta squared values are interpreted as the proportion of variance in a dependent variable explained by an independent variable, where 0.01=small, 0.06=medium, and 0.14=large. Hedge's *g* provides an unbiased measure of the standardised difference between means, where 0.20=small, 0.50=medium, and 0.80=large. Finally, for non-parametric tests, Pearson's *r* is interpreted as 0.10=small, 0.30=large, and 0.50=large (Cohen, 1988). Inferential statistics for non-theoretically relevant effects are provided in Appendix G.

Results

Demographics

Descriptive statistics for demographic variables are presented in Table 1. Results revealed small and non-significant differences in age, $t(35)=-0.73$, $p=.470$,

g=0.24, psychological distress (K10), *t*(35)=1.26, *p*=.215, *g*=0.41, and alcohol usage (AUDIT), *t*(35)=-1.20, *p*=.238, *g*=0.39, between the conditions.

Table 1.

Descriptive statistics for demographic variables for each condition.

	Control		Stress	
Variable	*M (SD)*	95% CI [*LL*, *UL*]	*M (SD)*	95% CI [*LL*, *UL*]
Age	21.74 (3.98)	[19.82, 23.66]	22.89 (5.52)	[20.14, 25.63]
K10	15.58 (4.67)	[13.33, 17.83]	13.83 (3.65)	[12.02, 15.65]
AUDIT	4.37 (3.02)	[2.91, 5.83]	5.67 (3.55)	[3.90, 7.43]

Note. CI = confidence interval; *LL* = lower limit; *UL* = upper limit.

Manipulation Checks

Subjective Units of Distress Scale (SUDS). Descriptive statistics for SUDS ratings are presented in Table 2. There were significant and large main effects of Time, $F(2.6, 91.4)=34.44$, $p<.001$, $\eta_p^2=.496$, and Condition, $F(1, 35)=30.28$, $p< .001$, $g=1.67$, and a significant and large Time x Condition interaction, $F(2.6, 91.4)=44.04$, $p<.001$, $\eta_p^2=.557$. Follow-up comparisons revealed that SUDS ratings were significantly greater for stressed participants than controls at 5- ($p<.001$, $g=3.93$) and 30-minutes post-stress manipulation ($p=.048$, $g=0.66$), but not at baseline ($p=.105$, $g=0.54$) or 45-minutes post-stress manipulation ($p=.528$, $g=0.21$).

However, significant Levene's tests indicated a violation of the assumption of homogeneity of variance at baseline and 5-minutes post-stress manipulation. Independent-samples Mann-Whitney U tests were therefore conducted at these time points. Results similarly identified a small, non-significant difference between stressed participants (*Mdn*=20.00, *IQR*=20.00) and controls (*Mdn*=10.00,

IQR=10.00) at baseline (*U*=214.00, *p*=.199, *r*=.22), but a large, significant difference between stressed participants (*Mdn*=60.00, *IQR*=27.50) and controls (*Mdn*=10.00, *IQR*=10.00) at 5-minutes post-stress manipulation (*U*=342.00, *p*<.001, *r*=.86).

Table 2.

Descriptive statistics for Subjective Units of Distress Scale (SUDS) ratings at each collection point.

	Control		Stress	
Time	*M (SD)*	95% CI [*LL, UL*]	*M (SD)*	95% CI [*LL, UL*]
Baseline	15.95 (10.16)	[11.05, 20.84]	24.72 (20.47)	[15.54, 34.90]
5	11.58 (6.25)	[8.57, 14.59]	61.81 (16.76)	[53.47, 70.14]
30	13.03 (10.23)	[8.10, 17.96]	20.97 (13.26)	[14.38, 27.57]
45	14.21 (10.84)	[8.99, 19.43]	16.53 (11.28)	[10.92, 22.14]

Note. CI = confidence interval; *LL* = lower limit; *UL* = upper limit.

Saliva Samples. One-sample *t*-tests between the mean recorded time of saliva sample measurements and their respective target times revealed that sample two (*M*=2’43”, *SD*=1’32”) was taken significantly earlier than the targeted five minute mark, *t*(35)=-8.91, *p*<.001, while sample three (*M*=34’33”, *SD*=2’54”) was taken significantly later than the targeted 30 minute mark, *t*(35)=9.40, *p*<.001. Mean collection time of sample four (*M*=45’46”, *SD*=3’5”) did not differ significantly from the targeted 45-minute mark, *t*(35)=1.49, *p*=.146.

Descriptive statistics for both conditions’ cortisol measurements are presented in Table 3. There were significant and large main effects of Time, *F*(1.3, 42.9)=8.04, *p*=.004, η_p^2=.191, and Condition, *F*(1, 34)=12.50, *p*=.001, *g*=1.15, and a significant and large Time x Condition interaction, *F*(1.3, 42.9)=26.22, *p*<.001, η_p^2

=.435. Follow-up comparisons revealed a non-significant difference in cortisol concentrations between conditions at baseline (p=.881, g=0.05). However, cortisol concentrations were significantly greater for the stress condition at 5- (p=.042, g=0.68), 30- (p<.001, g=1.58) and 45-minutes post-stress manipulation (p<.001, g=1.34).

However, significant Levene's tests indicated a violation of the assumption of homogeneity of variance at 30- and 45-minutes post-stress. Mann-Whitney U tests conducted at these time points identified large, significant differences between stressed participants (*Mdn*=1.95, *IQR*=1.57) and controls (*Mdn*=0.62, *IQR*=0.49) at 30-minutes (U=306.00, p<.001, r=.76), and between stressed participants (*Mdn*=1.59, *IQR*=1.18)) and controls (*Mdn*=0.57, *IQR*=0.32) at 45-minutes post-stress (U=288.50, p<.001, r=.067).

Table 3.

Descriptive statistics for salivary cortisol measurements (ng/mL) at each collection point.

	Condition			
	Control		Stress	
Time	*M (SD)*	95% CI [*LL, UL*]	*M (SD)*	95% CI [*LL, UL*]
Baseline	1.06 (0.56)	[0.78, 1.33]	1.03 (0.54)	[0.76, 1.30]
5	0.88 (0.54)	[0.61, 1.15]	1.31 (0.69)	[0.97, 1.65]
30	0.69 (0.39)	[0.50, 0.88]	2.31(1.36)	[1.64, 2.98]
45	0.66 (0.34)	[0.49, 0.83]	1.88 (1.21)	[1.28, 2.48]

Note. CI = confidence interval; *LL* = lower limit; *UL* = upper limit.

Behavioural Data

Reaction Time (ms). Cell means for reaction time are presented in Table 4. There was a small, significant main effect of Time, $F(1, 35)=11.70$, $p=.002$, $g=0.23$, and a large, significant main effect of Flanker Congruency, $F(1, 35)=477.43$, $p<.001$, $g=1.66$, such that participants had faster reaction times at post- ($M=515.31$, $SD=48.13$) than pre-stress manipulation ($M=503.44$, $SD=54.36$) and for congruent ($M=465.83$, $SD=42.98$) than incongruent flankers ($M=552.93$, $SD=59.09$), respectively. There was also a significant and large main effect of Cue, $F(1.6, 57.5)=321.09$, $p<.001$, $\eta_p^2=.902$, with pairwise comparisons revealing faster reaction times following spatial ($M=469.69$, $SD=56.51$) than central cues ($M=519.41$, $SD=48.17$) ($p<.001$, $g=0.93$), and following central cues than no cue ($M=540.04$, $SD=48.55$) ($p<.001$, $g=0.42$). The main effect of Condition was small and non-significant, $F(1,35)=0.47$, $p=.499$, $g=0.22$.

The hypothesised Time x Condition x Cue interaction was small and non-significant, $F(1.6, 55.7)=0.11$, $p=.857$, $\eta_p^2=.003$. Planned follow-up comparisons revealed non-significant Cue x Condition interactions at pre-, $F(1.8, 63.9)=0.28$, $p=.735$, $\eta_p^2=.008$, and post-stress manipulation, $F(1.8, 62.5)=0.30$, $p=.715$, $\eta_p^2=.009$, with non-significant differences between conditions for all Cue types pre- and post-stress manipulation (see Table 5). The hypothesised Time x Condition x Flanker Congruency interaction was also small and non-significant, $F(1, 35)=0.41$, $p=.524$, $\eta_p^2=.012$. Follow-up comparisons revealed non-significant Condition x Flanker Congruency interactions at pre-, $F(1, 35)=0.22$, $p=.646$, $\eta_p^2=.006$, and post-stress manipulation, $F(1, 35)=0.02$, $p=.881$, $\eta_p^2=.001$, with non-significant differences

Table 4.

Cell means for reaction time (ms) for all Condition, Time, Cue, and Flanker Congruency conditions.

			Condition			
			Control		Stress	
Time	Flanker	Cue	*M (SD)*	95% CI *[LL, UL]*	*M (SD)*	95% CI *[LL, UL]*
Pre	Congruent	No	507.49 (35.52)	[490.37, 524.61]	497.90 (45.49)	[475.27, 520.52]
		Central	481.69 (28.71)	[467.85, 495.53]	468.01 (48.09)	[444.10, 491.93]
		Spatial	439.14 (38.96)	[420.37, 457.92]	425.21 (47.89)	[401.40, 449.03]
	Incongruent	No	588.47 (41.39)	[568.51, 608.42]	582.08 (63.90)	[550.31, 613.86]
		Central	581.69 (43.61)	[560.67, 602.71]	574.72 (65.03)	[542.38, 607.06]
		Spatial	524.34 (47.96)	[501.22, 547.45]	513.02 (79.09)	[473.69, 552.36]
Post	Congruent	No	500.42 (41.57)	[480.38, 520.45]	492.98 (55.67)	[465.30, 520.66]
		Central	470.75 (39.02)	[451.94, 489.56]	456.77 (48.20)	[432.80, 480.74]
		Spatial	432.30 (44.89)	[410.67, 453.94]	417.28 (56.55)	[389.16, 445.40]
	Incongruent	No	580.41 (42.61)	[559.87, 600.95]	570.56 (70.12)	[535.68, 605.43]
		Central	566.79 (49.83)	[542.78, 590.81]	554.87 (67.49)	[521.30, 588.43]
		Spatial	504.71 (53.90)	[478.73, 530.69]	493.47 (82.64)	[452.38, 534.56]

Note. CI = confidence interval; *LL* = lower limit; *UL* = upper limit.

between conditions for congruent and incongruent stimuli at each time point (see Table 5). The magnitude of the alerting, orienting, and congruency effects therefore did not differ between conditions (see Figure 3).

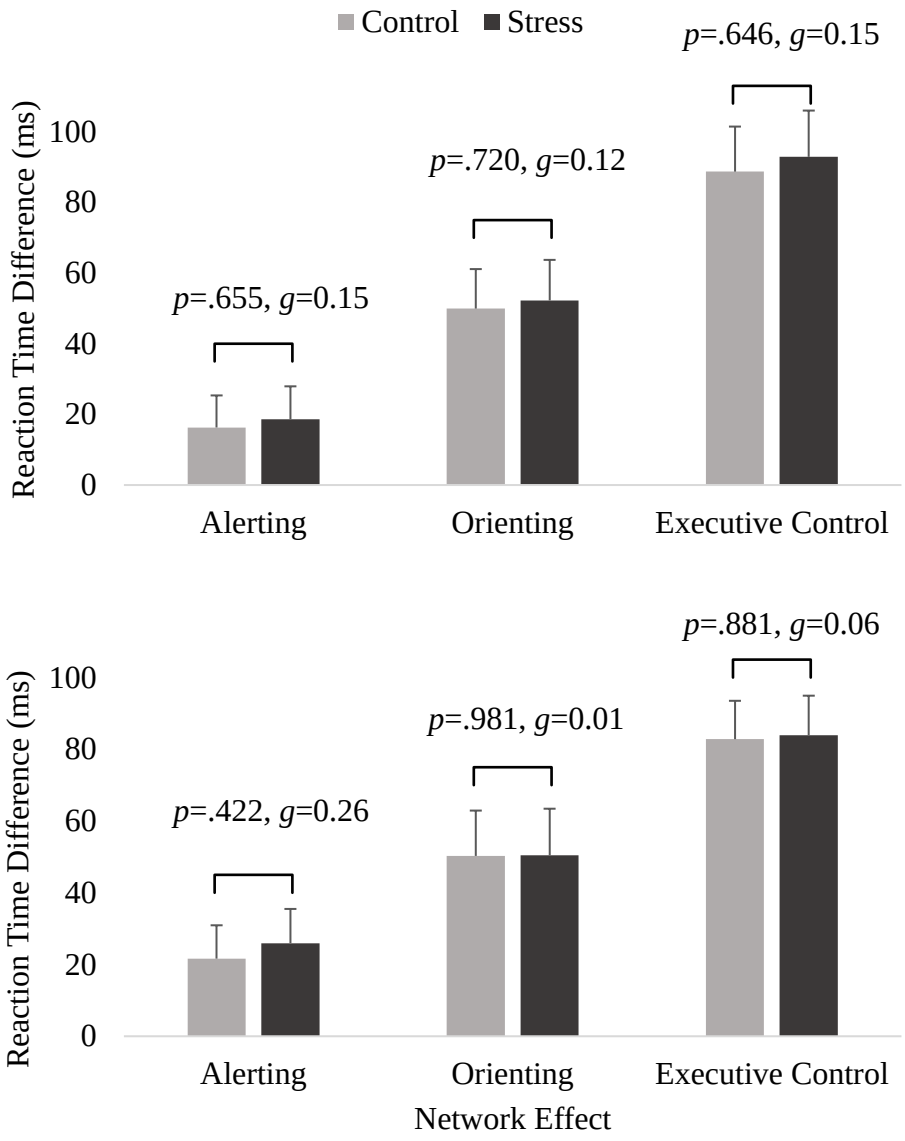


Figure 3. Alerting (central - no cue), orienting (spatial - central cue), and executive control (incongruent – congruent flakers) network effects for both conditions pre- (above) and post- (below) stress manipulation. Significance values for the alerting and orienting effects came from Independent-samples *t*-tests with 35 degrees of freedom. Error bars denote 95% confidence intervals.

Table 5.

Pairwise comparisons between conditions for measures of reaction time (ms).

Time	Effect	*p*	*g*	95%CI[*LL*, *UL*]
Pre	No Cue	0.596	0.17	[-38.34, 22.36]
	Central Cue	0.496	0.22	[-40.78, 20.14]
	Spatial Cue	0.472	0.23	[-47.83, 22.58]
	Congruent Flankers	0.351	0.30	[-39.01, 14.21]
	Incongruent Flankers	0.660	0.14	[-45.82, 29.38]
Post	No Cue	0.618	0.16	[-43.54, 26.25]
	Central Cue	0.438	0.25	[-46.45, 20.54]
	Spatial Cue	0.504	0.22	[-52.64, 26.38]
	Congruent Flankers	0.432	0.26	[-43.14, 18.85]
	Incongruent Flankers	0.585	0.18	[-51.51, 29.49]

Note. CI = confidence interval of mean difference; *LL* = lower limit; *UL* = upper limit.

Accuracy (%). Cell means for accuracy are presented in Table 6. There was a significant and large main effect of Flanker Congruency, $F(1, 35)=139.73$, $p<.001$, $g=1.64$), such that accuracy was greater for congruent ($M=97.70$, $SD=2.00$) than incongruent flankers ($M=92.09$, $SD=4.29$). There was also a significant and large main effect of Cue, $F(1.8, 63.5)=16.73$, $p<.001$, $\eta_p^2=.323$, such that accuracy was greater following spatial cues ($M=96.07$, $SD=3.35$) than central cues ($M=94.44$, $SD=3.05$) ($p<.001$, $g=0.50$) or no cue ($M=94.16$, $SD=3.41$) ($p<.001$, $g=0.55$), but did not differ between central cues and no cue ($p=1.000$, $g=0.09$). The main effects of Condition, $F(1, 35)=0.22$, $p=.645$, $g=0.15$, and Time, $F(1, 35)=0.51$, $p=.480$, $g=0.10$, were both small and non-significant.

The hypothesised Time x Congruency x Condition interaction was small and non-significant, $F(1, 35)=0.20$, $p=.657$, $\eta_p^2=.006$ (see Figure 4 for depiction of the executive control effects at pre- and post-stress). Planned comparisons revealed a small and non-significant Condition x Flanker Congruency interaction pre-stress manipulation, $F(1, 35)=0.48$, $p=.827$, $\eta_p^2=.001$, with non-significant differences between conditions for congruent *(*$p=.561$, $g=0.19$) and incongruent stimuli ($p=.938$, $g=0.03$). Following the stress manipulation, the Condition x Flanker Congruency interaction was also small and non-significant, $F(1, 35)=0.34$, $p=.854$, $\eta_p^2=.001$, with non-significant differences between conditions for congruent ($p=.498$, $g=0.22$) and incongruent stimuli ($p=.627$, $g=0.16$).

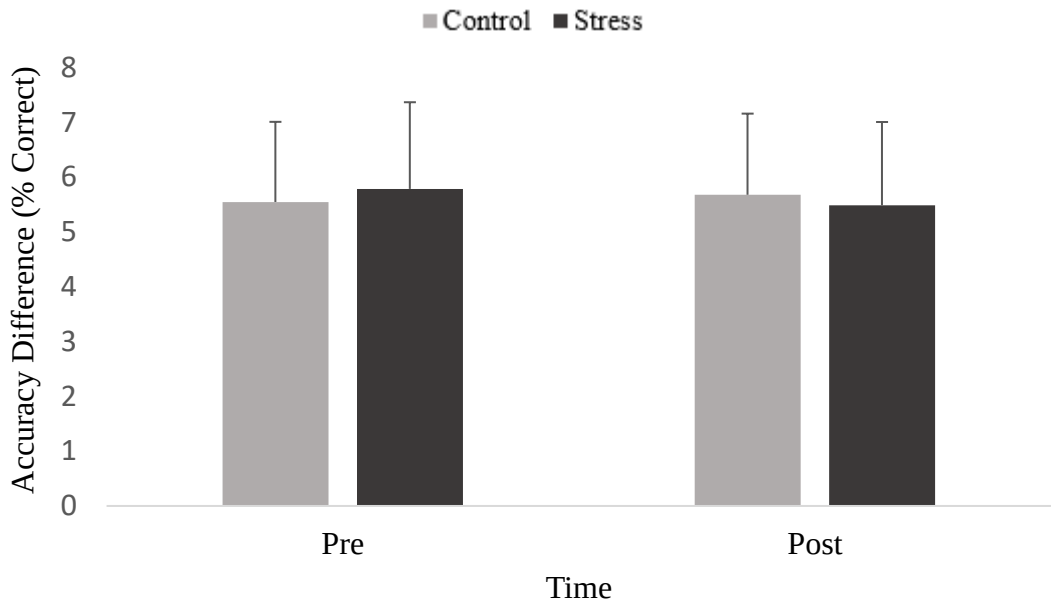


Figure 4. Executive control effect (congruent – incongruent) for accuracy (% correct) at pre- and post-stress manipulation for each condition. Error bars denote 95% confidence intervals.

Table 6.

Cell means for accuracy (% correct) for all Condition, Time, Cue, and Flanker Congruency conditions.

			Condition			
			Control		Stress	
Time	Flanker	Cue	*M (SD)*	95% CI *[LL, UL]*	*M (SD)*	95% CI *[LL, UL]*
Pre	Congruent	No	96.71 (3.10)	[95.22, 98.20]	97.57 (2.55)	[96.30, 98.84]
		Central	98.09 (2.26)	[97.00, 99.18]	98.06 (1.50)	[97.31, 98.80]
		Spatial	98.36 (2.21)	[97.29, 99.42]	98.61 (1.41)	[97.91, 99.31]
	Incongruent	No	91.58 (5.20)	[89.07,94.09]	90.35 (5.65)	[87.54, 93.16]
		Central	91.51 (6.78)	[88.25, 94.78]	91.53 (3.14)	[89.97, 93.09]
		Spatial	93.42 (6.55)	[90.27, 96.58]	95.00 (3.12)	[93.45, 96.55]
Post	Congruent	No	97.12 (2.98)	[95.67, 98.54]	97.57 (2.33)	[96.41, 98.73]
		Central	97.70 (2.95)	[96.27, 99.12]	97.50 (2.50)	[96.26, 98.74]
		Spatial	96.84 (4.13)	[94.85, 98.83]	98.33 (2.88)	[96.90, 99.76]
	Incongruent	No	90.59 (6.37)	[87.52, 93.66]	91.81 (5.00)	[89.32, 94.29]
		Central	90.99 (5.78)	[88.20, 93.77]	90.14 (5.30)	[87.50, 92.78]
		Spatial	93.03 (6.90)	[89.70, 96.35]	95.00 (2.64)	[93.69, 96.31]

Note. CI = confidence interval; *LL* = lower limit; *UL* = upper limit.

Electrophysiological Data

N1 Amplitude (μV). Figure 5 shows the grand averaged ERP waveforms for the target-locked N1 at the Oz electrode. Cell means for Oz electrode are presented in Table 7.

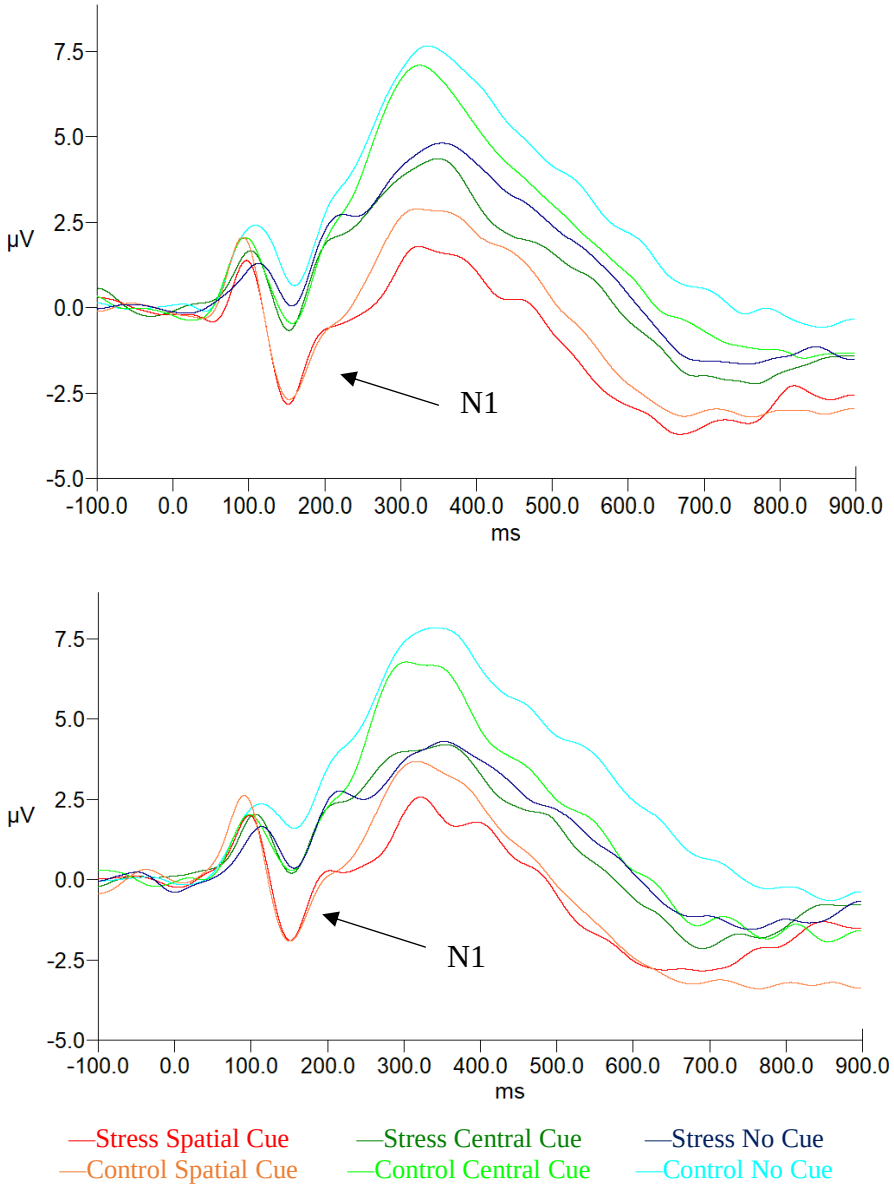


Figure 5. Grand averaged ERP waveforms for target-locked N1 at the Oz electrode, pre- (above) and post- (below) stress manipulation.

Table 7.

Cell means for N1amplitude (μV) at the Oz electrode for all Condition, Time, Cue, and Flanker Congruency conditions.

			Condition			
			Control		Stress	
Time	Flanker	Cue	*M (SD)*	95% CI *[LL, UL]*	*M (SD)*	95% CI *[LL, UL]*
Pre	Congruent	No	-0.95 (2.51)	[-2.16, 0.25]	-1.19 (2.54)	[-2.45, 0.08]
		Central	-1.85 (2.69)	[-3.15, -0.56]	-1.99 (3.05)	[-3.50, -0.47]
		Spatial	-3.99 (3.12)	[-5.49, -2.48]	-2.64 (2.81)	[-5.04, -2.25]
	Incongruent	No	-0.70 (2.21)	[-1.76, 0.37]	-1.03 (2.57)	[-2.31, 0.25]
		Central	-1.71 (3.23)	[-3.27, -0.16]	-1.68 (3.14)	[-3.24, -0.12]
		Spatial	-3.85 (3.18)	[-5.38, -2.32]	-4.45 (3.18)	[-6.03, -2.86]
Post	Congruent	No	-0.03 (2.48)	[-1.22, 1.17]	-0.62 (2.86)	[-2.05, 0.80]
		Central	-1.37 (3.64)	[-3.12, 0.39]	-0.89 (2.44)	[-2.11, 0.32]
		Spatial	-3.03 (3.26)	[-4.60, -1.46]	-2.90 (2.97)	[-4.38, -1.43]
	Incongruent	No	0.41 (2.39)	[-0.74, 1.57]	-1.00 (3.02)	[-2.50, 0.50]
		Central	-0.85 (2.94)	[-2.26, 0.57]	-1.19 (2.70)	[-2.53, 0.16]
		Spatial	-3.31 (4.09)	[-5.28, -1.34]	-3.25 (2.66)	[-4.58, -1.93]

Note. CI = confidence interval; *LL* = lower limit; *UL* = upper limit.

Results revealed a significant and large main effect of Cue, $F(1.6, 57.6)=42.75$, $p<.001$, $\eta_p^2=.550$, with pairwise comparisons indicating that N1 amplitude was significantly greater following central cues (M=-1.44, SD=2.84) relative to no cue (M=-0.64, SD=2.43) (p=.012, g=0.30), and following spatial cues (M=-3.55, SD=3.05) relative to central cues ($p<.001$, g=0.70). There was also a small and significant main effect of Time, $F(1, 35)=23.44$, $p<.001$, g=0.28, such that N1 amplitude was greater pre (M=-2.25, SD=2.53) than post stress manipulation (M=-1.50, SD=2.63). The main effect of Condition was small and non-significant, $F(1, 35)=0.07$, p=.796, g=0.08. The hypothesised Time x Condition x Cue interaction was moderate but non-significant, $F(1.9, 68.12)=2.33$, p=.107, $\eta_p^2=.062$, with small, non-significant Condition x Cue interactions at pre-, $F(1.8, 63.0)=0.06$, p=.927, $\eta_p^2=.002$, and post-stress manipulation, $F(1.7, 58.2)=1.55$, p=.223, $\eta_p^2=.042$. Pairwise comparisons revealed non-significant differences between conditions for all Cue types pre- and post-stress manipulation (see Table 8).

Table 8.

Pairwise comparisons between conditions for measures of N1 amplitude (μV).

Time	Effect	*p*	*g*	95%CI[*LL*, *UL*]
Pre	No Cue	.720	0.12	[-1.87, 1.30]
	Central Cue	.958	0.02	[-2.02, 1.92]
	Spatial Cue	.898	0.04	[-2.14, 1.89]
Post	No Cue	.248	0.38	[-2.74, 0.73]
	Central Cue	.946	0.02	[-1.87, 2.00]
	Spatial Cue	.929	0.03	[-2.05, 2.23]

Note. CI=confidence interval of mean difference; *LL* = lower limit; *UL* = upper limit.

P3 Amplitude (µV). Figure 6 shows the grand averaged ERP waveforms for the target-locked P3 at the Fz electrode. Cell means for the Fz electrode are presented in Table 9.

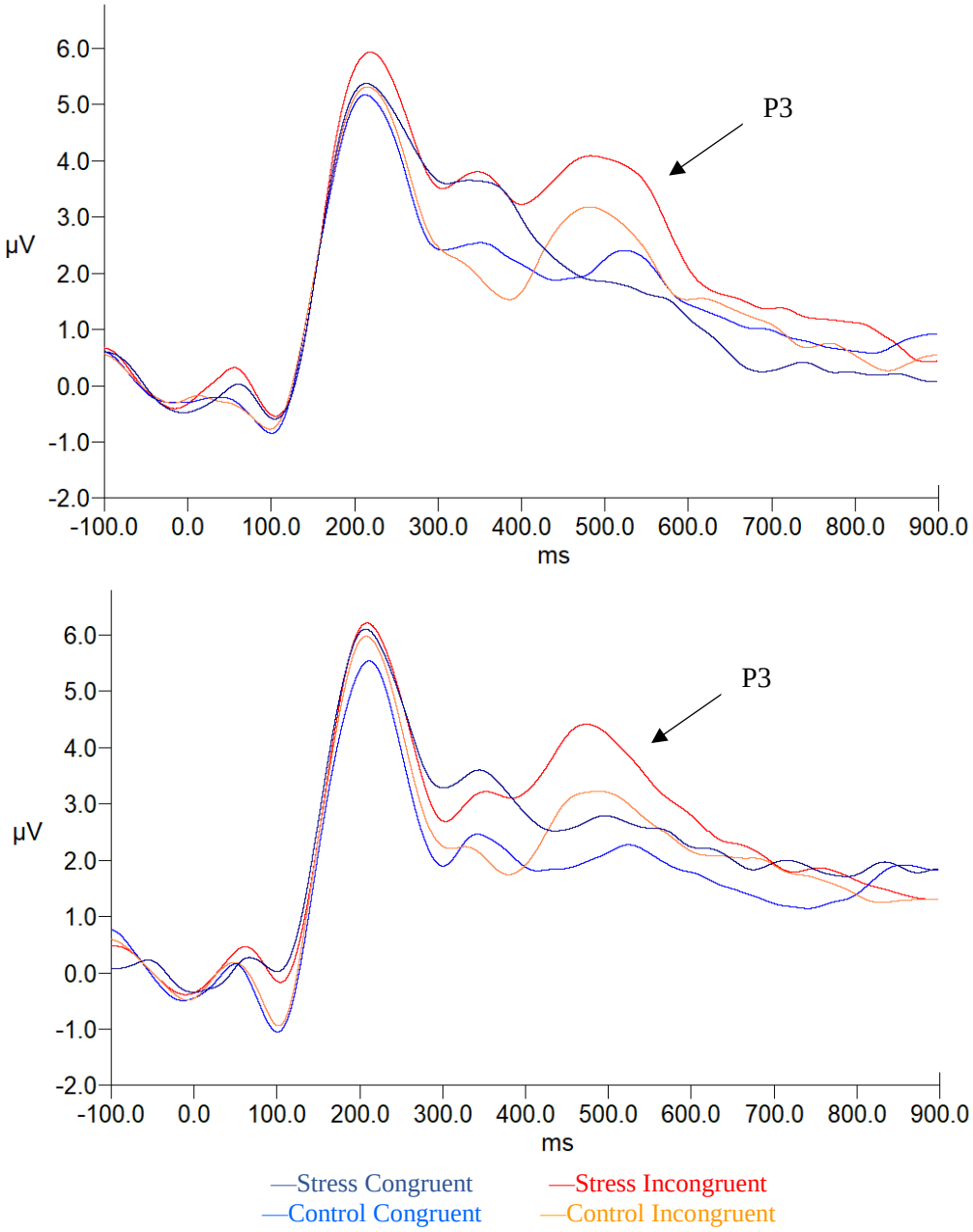


Figure 6. Grand averaged ERP waveforms for the target-locked P3 at the Fz electrode, pre- (above) and post- (below) stress manipulation.

Table 9.

Cell means for P3 amplitude (μV) at the Fz electrode for all Condition, Time, Cue, and Flanker Congruency conditions

			Condition			
			Control		Stress	
Time	Flanker	Cue	*M (SD)*	*95% CI [LL, UL]*	*M (SD)*	*95% CI [LL, UL]*
Pre	Congruent	No	3.33 (3.30)	[1.70, 4.97]	3.60 (4.57)	[1.33, 5.88]
		Central	5.88 (2.93)	[4.47, 7.30]	7.22 (3.62)	[5.42, 9.02]
		Spatial	6.38 (2.62)	[5.17, 7.59]	7.07 (3.46)	[5.35, 8.79]
	Incongruent	No	3.60 (3.40)	[1.96, 5.24]	4.18 (4.07)	[2.15, 6.20]
		Central	6.74(2.39)	[5.59, 7.90]	8.41 (3.22)	[6.81, 10.01]
		Spatial	6.51 (2.62)	[5.25, 7.78]	7.59 (5.52)	[5.83, 9.34]
Post	Congruent	No	2.43 (3.30)	[0.84, 4.03]	2.74 (3.87)	[0.82, 4.67]
		Central	6.29 (2.51)	[5.08, 7.50]	7.99 (2.79)	[6.60, 9.38]
		Spatial	6.91 (2.67)	[5.62, 8.20]	8.56 (3.81)	[6.67, 10.46]
	Incongruent	No	3.05 (3.12)	[1.54, 4.55]	3.40 (4.01)	[1.41, 5.40]
		Central	6.99 (2.76)	[5.66, 8.32]	8.65 (2.69)	[7.31, 9.99]
		Spatial	7.75 (2.73)	[6.43, 9.07]	8.71 (3.48)	[6.97, 10.44]

Note. CI = confidence interval; *LL* = lower limit; *UL* = upper limit.

There was a significant and large main effect of Flanker Congruency, $F(1, 35)=12.08$, $p=.001$, $g=0.24$, with P3 amplitude being greater for incongruent ($M=6.30$, $SD=2.41$) than congruent flankers ($M=5.70$, $SD=2.54$). The main effects of Time, $F(1, 35)=1.71$, $p=.199$, $g=0.10$ and Condition, $F(1, 35)=1.64$, $p=.208$, $g=0.40$, were both small and non-significant. The hypothesised Time x Flanker Congruency x Condition interaction was small and non-significant, $F(1, 35)=0.70$, $p=.408$, $\eta_p^2=.020$. Planned comparisons revealed a small and non-significant Flanker Congruency x Condition interaction pre-stress manipulation, $F(1, 35)=0.50$, $p=.485$, $\eta_p^2=.014$, with small congruency P3 effects for stressed participants ($M_{difference}=0.77\mu V$, $p=.034$, $g=0.27$) and controls ($M_{difference}=0.42\mu V$, $p=.218$, $g=0.15$). Similarly, there was a small and non-significant Flanker Congruency x Condition interaction post stress manipulation, $F(1, 35)=0.22$, $p=.640$, $\eta_p^2=.006$, with small congruency P3 effects for stressed participants ($M_{difference}=0.49\mu V$, $p=.168$, $g=0.18$) and controls ($M_{difference}=0.72\mu V$, $p=.041$, $g=0.27$) (see Figure 7).

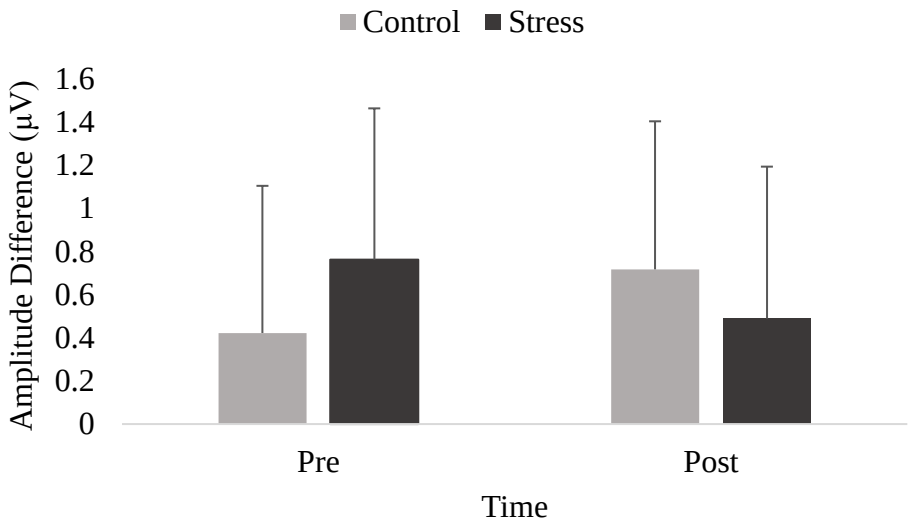


Figure 7. Congruency P3 effect (incongruent-congruent) at pre- and post-stress manipulation for each condition. Error bars denote 95% confidence intervals.

Discussion

The present study aimed to examine the influence of acute stress on behavioural and electrophysiological measures of human attentional networks. The hypotheses that following stress manipulation, stressed participants would display faster reaction times and greater N1 amplitude for central and spatial cues, slower reaction times and/or worse accuracy for incongruent flankers, and reduced congruency effects for P3 amplitude relative to controls were all unsupported. Behaviourally, stressed participants and controls displayed comparable reaction time and accuracy for all Cue and Flanker Congruency conditions at both pre- and post-stress manipulation. Electrophysiologically, stressed participants and controls recorded similar amplitude modulations for each Cue condition at pre- and post-stress manipulation, while the congruency P3 effect was not significantly reduced for stressed participants relative to controls. Despite these findings, both manipulation checks indicated that the MAST was successful in inducing a psychological and biochemical stress response.

Successful Stress Manipulation

As mentioned, both manipulation checks indicated a successful stress induction. SUDS ratings collected immediately following the stress manipulation revealed that the MAST induced significantly greater levels of distress than did the MAST-placebo, and that this effect was very large. Following recompletion of the ANT, stressed participants still recorded significantly greater levels of distress than controls, although the magnitude of the effect was comparable to the baseline measurement. By time four, only a negligible difference was present between conditions.

Salivary cortisol measurements further revealed that stressed participants had significantly higher cortisol concentrations than controls at all stages following

completion of the MAST/MAST-placebo, but not at baseline. In line with previous findings, peak salivary cortisol concentration occurred approximately 30 minutes post-stress (Gagnon & Wagner, 2016). However, collection of sample three was delayed significantly beyond target collection time. Peak cortisol concentrations may therefore be underestimates. Collectively, these results confirm that the MAST acted as a successful stress manipulation, as described by Smeets et al. (2012).

No Differential Effect of Acute Stress on Attention

Previous literature has identified a differential effect of acute stress on cognitive functions such as attention (Arnsten, 2009). It is suggested that in order to facilitate threat detection, activation of the stress response weakens prefrontal cortex function while strengthening primary sensory cortices, biasing cognition to enhance processing of information related to the stressor (Arnsten, 2009; Shields et al., 2016). Specifically, stress-induced increases in dopamine and cortisol have been found to impair top-down cognitive functions (Lupien et al., 2002; Matuszewich et al., 2014), while increases in noradrenaline and acetylcholine alter neuronal firing by increasing sensory gain (Aston-Jones & Cohen, 2005a; Picciotto et al., 2012). Considering this literature, we utilised the ANT as a measure of attentional processing as the neurotransmitters and brain regions associated with the attentional networks overlap considerably with the acute stress response.

Analysis of behavioural and electrophysiological data revealed the expected patterns of basic task modulation as found in previous studies using the ANT (Fan et al., 2002; Neuhaus et al., 2010). There were significant main effects of Cue and Flanker Congruency for reaction time, such that reaction time improved with cue informativity and for congruent relative to incongruent flankers. Similarly, accuracy was greater for

congruent than incongruent flankers. Electrophysiologically, N1 amplitude increased with cue informativity, while there was a significant effect of Flanker Congruency on P3 amplitude. These findings indicate that the lack of Condition effects was not due to an inability to activate attentional networks. Rather, our results are consistent with patterns of performance predicted by attentional network theory (Petersen & Posner, 2012).

While overall performance in the current study was as expected, the lack of hypothesised interactions for all dependent variables suggests that acute stress induction had no discernible influence on measures of human attentional networks. While these findings do not support the hypotheses, they are congruent with some findings from the previous literature. Both Dierolf et al. (2017) and Larra, Pramme, Schachinger, and Frings (2016) reported non-significant effects of stress on measures of reaction time and accuracy using the SECPT and the Cold Pressor Test, respectively. Similarly, Shackman et al. (2011) found non-significant behavioural effects using an electric shock stress induction method and a flanker-style speeded response task. Thus, while the behavioural results from the current study were not hypothesised, they demonstrate both support and disagreement with previous literature (for opposing results, see: Jiang et al., 2017; Qi et al., 2018).

However, the lack of effect of stress on electrophysiological measures of attention in the current study is largely inconsistent with previous findings. Both Dierolf et al. (2017) and Shackman et al. (2011) identified greater N1 and reduced P3 amplitude following stress, despite a lack of behavioural differences. Other studies have demonstrated similar amplitude modulations of these or related components (Ermutlu, Karamursel, Ugur, Senturk, & Gokhan, 2005; Jiang et al., 2017; Qi, Gao, & Liu, 2017), indicating that neural markers of attention are ostensibly more susceptible to the effects

of acute stress than behavioural measures. The results of the present study are therefore unexpected based on previous literature.

Possible Explanations for Null Results

In explaining their null behavioural results, Dierolf et al. (2017) cited differences in stress induction and behavioural testing paradigms relative to other studies. Indeed, the substantial methodological differences between existing studies of acute stress and attention make a comparison of results challenging. This is an important consideration in the current study, as it is the first to our knowledge to utilise the MAST/ANT combination. As such, the present results may relate to the inability of the MAST to induce changes in attentional processing (i.e. the magnitude of the induced stress response) and/or the inability of the ANT to capture these changes.

Regarding the former possibility, while both manipulation checks indicated that the MAST was successful, participants in both conditions recorded lower mean distress ratings following recompletion of the ANT than at baseline, suggesting the subjective effects of the MAST may not have been strong enough to persist throughout the duration of the second ANT. Furthermore, although salivary cortisol was significantly elevated for stressed participants at all time points following the MAST, cortisol has not been previously linked to the functioning of the attentional networks (Petersen & Posner, 2012). This suggests that elevated cortisol alone is insufficient to affect performance on the ANT, as previous studies have shown a dissociable effect of cortisol on aspects of cognition (Shields et al., 2016). However, activation and deactivation of the sympathetic branch of the acute stress response occurs on a much faster time scale than that of the neuroendocrine system, with Smeets et al. (2012) finding salivary biomarkers of noradrenaline concentrations to be similar to baseline levels at 10 minutes following the

MAST. As noradrenaline is directly related to the ANT, it is possible that the acute biochemical effects of stress may have also been mitigated by task length.

To test for this possibility, a supplementary analysis was run on reaction time data following the stress manipulation (see Appendix H for statistical output). An additional variable of trial Block was created for this analysis, such that stimuli were coded according to their order of appearance. As there were 80 trials per Cue by Flanker Congruency condition, stimuli were averaged into four blocks of 20 (i.e. the first 20 of each condition to appear were included in block one, the next 20 in block two, etc.). This allowed us to determine if the effects of Condition varied depending on when stimuli were presented. If this were true, it may be expected that the hypothesised interactions would be present for stimuli appearing early in the task when subjective and biochemical stress levels were highest. However, the results revealed no significant interactions involving Condition or Block, tentatively ruling out the possibility of task length mitigating the effects of stress (although this analysis was not feasible for electrophysiological data).

Despite these results, the procedural order of stress manipulations and attention tasks is undoubtedly a key methodological component of acute stress studies. For example, rather than separating the stress manipulation and attention task, Qi et al. (2018) employed a combined procedure whereby participants performed both simultaneously. This potentially allowed the acute effects of stress to be captured more effectively. Conversely, the attention task utilised by Sanger et al. (2014) was separated from the stress manipulation by 20 minutes to capture peak cortisol concentrations during task performance. These methodological differences may help explain the discrepancy of results between the current and previous studies.

Another salient methodological consideration is the coding of participants according to the magnitude of their stress response. Both Dierolf et al. (2017) and Jiang et al. (2017) separated stressed participants into high and low cortisol responders according to a median split of cortisol reactivity (i.e. peak cortisol minus baseline). Dierolf et al. (2017) subsequently identified larger P3 amplitude reductions post-stress in high cortisol responders. Alternatively, Sanger et al. (2014) used a correlational analysis to identify that salivary cortisol concentration was negatively associated with P3 amplitude for stressed participants. These findings suggest that individual variability in the stress response may account for the lack of Condition effects in the present study.

To test for this possibility, supplementary regression analyses were run between stressed participants' cortisol reactivity and reaction time/P3 amplitude for incongruent stimuli post-stress (see Appendix H for statistical output). These dependent variables were chosen due to their association with the executive control network and the hypothesised detrimental effect of cortisol on executive functions, while regression was chosen to avoid the loss of power associated with conducting a median split (Iacobucci, Posavac, Kardes, Schneider, & Popovich, 2015). As with the Block analysis, however, there were no significant associations between participants' cortisol reactivity and measures of executive function, suggesting that the observed null results were not due to variability in the magnitude of the acute stress response.

Given these findings, it is unlikely that the results are related to properties of the MAST (i.e. the magnitude or duration of stress induction). Consequently, it may be that the ANT itself is insensitive to the effects of stress. However, using null hypothesis significance testing it is not possible to distinguish task insensitivity (i.e. true null results) from that of data insensitivity (e.g., lack of power) as statistical non-significance

cannot quantify evidence in favour of the null hypothesis (Dienes, 2014). Larra et al. (2016) similarly identified this issue, reporting null effects on measures of reaction time and accuracy despite a successful stress manipulation. Larra et al. (2016) therefore conducted an additional Bayesian analysis to assist with data interpretation. Rather than significance values, Bayesian analysis produces *Bayes factors* capable of quantifying statistical evidence for a model given the collected data (Wagenmakers et al., 2018). Thus, in line with Larra et al. (2016), a supplementary Bayesian analysis was also conducted on data from the current study.

JASP Version 0.9.0.0 was used to run a Bayesian mixed ANOVA with default priors on the same variables included in the primary analyses described above. To determine the evidence against the hypothesised interaction effects, the inclusion Bayes factor ($BF_{inclusion}$) across matched models was computed (i.e. the ratio of the evidence for models including the effect of interest, but not higher-order interactions with the effect of interest, to the evidence for models without the effect of interest) (Alilović, Timmermans, Reteig, van Gaal, & Slagter, in press). Using the classification scheme outlined by Wagenmakers et al. (2018), $BF_{inclusion}$ values were categorised as 1.00-0.33=anecdotal evidence, 0.33-0.10=moderate evidence, 0.10-0.03=strong evidence, 0.03-0.01=very strong evidence, and <0.01=extreme evidence for the null hypothesis of not including an effect. The reciprocal of these values yields the classification for including an effect (e.g., 1/0.10 = 10; moderate-strong evidence).

Results indicated moderate evidence against inclusion of the hypothesised Time by Condition by Flanker Congruency interactions for reaction time ($BF_{inclusion}$=0.163), accuracy ($BF_{inclusion}$=0.202), and P3 amplitude ($BF_{inclusion}$=0.308), and moderate/strong evidence against the Time by Condition by Cue interactions for N1 amplitude

($BF_{inclusion}$=0.204) and reaction time ($BF_{inclusion}$=0.067). Indeed, there was no supporting evidence for including any effect involving Condition for any dependent variable (see Appendix I). Conversely, extreme support was found for including the main effect of Cue for both reaction time and N1 amplitude, while the main effect of Flanker Congruency received extreme support in the reaction time and accuracy analyses, and moderate support in the P3 amplitude analysis. These results confirm those of the primary analysis in finding evidence of the expected basic task modulations, while also finding moderate evidence for a null effect of acute stress on ANT performance.

The explanation of a null effect in the current study is plausible for several reasons. Firstly, while acute stress is known to influence vigilance (van Marle, Hermans, Qin, & Fernandez, 2009), attentional orienting (Pilgrim, Marin, & Lupien, 2010), and executive functions (Shields et al., 2016), all of which are directly relevant to human attentional networks, properties of the ANT itself may have prevented these effects from emerging in the present study. For example, stress is suggested to bias attention towards bottom-up stimulus processing, such that exogenous (cf. top-down, endogenous) attention is dominant (Elling et al., 2011). However, as all cues in the ANT are valid, this involves both endogenous and exogenous attention (Roca, Castro, Lopez-Ramon, & Lupianez, 2011). Thus, the effect of stress on exogenous attention is not completely discernible using the ANT, and the role of executive functions in endogenous orienting is confounded if stress impairs executive control. These factors may have concealed the effects of stress on the orienting network.

Similarly, while stress may stimulate noradrenaline release and therefore increase alertness, alertness itself can be subdivided into *phasic* and *tonic* components relating to different firing rates of the locus coeruleus (Aston-Jones & Cohen, 2005a).

Phasic firing is associated with task relevant behavioural responses and is tested by the central/double cue ANT conditions. Contrarily, tonic firing refers to baseline firing and is somewhat assessed by the no cue condition (Posner, 2008). As stress has been shown to shift noradrenergic firing towards greater tonic activity (Hermans et al., 2011), it is possible that stress would have a greater effect on measures of tonic than phasic alertness. However, it has been argued that the no cue condition provides an inadequate measure of tonic alertness as it is assessed indirectly (Roca et al., 2011). Thus, if there was a differential effect of stress on tonic versus phasic alerting in the current study, the ANT may have again been unable to capture it.

Finally, given the diversity of cognitive abilities categorised as executive functions and the necessarily narrow focus of any single task, the particular aspects of executive function assessed by the ANT may not be influenced by acute stress. In describing the ANT, Fan et al. (2005) identified the incongruent flankers as introducing response conflict, thus requiring inhibition of irrelevant information (i.e. cognitive inhibition) and/or suppression of a response (i.e. response inhibition) (Kopp, Rist, & Mattler, 1996). Relative to other executive abilities, such as working memory, the effect of stress on inhibitory functions is less clear. Indeed, a meta-analysis by Shields et al. (2016) suggested that acute stress may improve response inhibition while impairing cognitive inhibition. Others, however, have found that acute stress narrows attentional focus to improve inhibition of irrelevant information, but only under conditions of low perceptual load (Sato, Takenaka, & Kawahara, 2012). These findings highlight the nuanced effects of stress on executive functions, leaving open the possibility of null results on ANT performance.

Limitations and Directions for Future Research

While the results of the current study suggest that the ANT may be insensitive to the effects of acute stress, this conclusion is limited by several factors. Firstly, we were unable to collect measures of sympathetic nervous system activation as has been done in previous studies (e.g., Larra et al., 2016). Thus, one of the two major sub-responses to acute stress, and a key theoretical component of the current study, was unable to be quantified. While salivary cortisol serves as a useful manipulation check, it may not be a relevant biomarker for attentional network function as described above. The collection of salivary alpha-amylase would therefore be of particular interest in future studies, as it is an indirect measure of noradrenaline (Nater & Rohleder, 2009). Thus, it may serve as both a manipulation check and a theoretically relevant biomarker of the alerting network (Petersen & Posner, 2012).

A second limitation is the potential influence of hormonal contraceptives and menstrual cycle phase as confounding variables. In previous research, women in the luteal phase demonstrated larger salivary cortisol responses than women in the follicular phase of their menstrual cycle or those using oral contraceptives (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). Greater noradrenaline reactivity following stress has also been observed for women in the luteal phase (Childs, Dlugos, & De Wit, 2010). Furthermore, oral contraceptive use is suggested to influence cognitive abilities such as spatial attention (Gogos, Wu, Williams, & Byrne, 2014). As such, stress responses and attentional functioning may have been moderated by these factors. While information on the menstrual cycle phase and contraceptive use were collected, these data were self-reported and lacked precision for statistical analysis. Additionally, sample sizes were insufficient for analyses with contraceptive use as a categorical variable.

Future studies should therefore aim to collect high precision data on these variables and increase sample sizes to control for their influence.

Finally, as specific properties of the ANT may have prevented stress effects from emerging, the use of alternate versions of the ANT is a promising area for future research. For example, Callejas et al. (2005) developed a revised version of the ANT to differentiate exogenous from endogenous attention and reduce the overlap between orienting and executive networks. This version is known as the Attention Network Test for Interaction (ANTI) and involves both valid and invalid cues. Roca et al. (2011) subsequently extended the ANTI to involve a secondary vigilance task, providing a direct measure of tonic alertness in addition to the standard scores. While these alternate versions of the ANT are less well researched, they may reveal the effects of acute stress not discernible using the standard ANT.

Summary and Conclusions

The current study aimed to examine the influence of acute stress on behavioural and electrophysiological markers of human attentional networks. Despite a successful stress induction, acute stress was found to have no effect on measures of reaction time, accuracy, or amplitude of the N1 and P3 ERP components. These null findings were subsequently confirmed by a Bayesian analysis, indicating moderate evidence against the inclusion of the hypothesised interaction effects. However, all expected basic task modulations were observed, indicating that the null findings were not due to an inability to activate attentional networks.

In identifying possible explanations for these results, supplementary analyses tentatively ruled out task duration mitigating the effects of acute stress and individual variability in the neuroendocrine stress response. Our results therefore suggest that

acute stress exerts no discernible influence on attentional networks, as measured by the ANT. As there is considerable evidence for a genuine effect of stress on attentional processing, the results likely relate to properties of the ANT itself, such as an inability to specifically measure exogenous attentional orienting and tonic alertness. Future research may address these issues by employing revised versions of the ANT designed to overcome these issues. Therefore, given the inability to measure and control for key components and confounding variables relevant to the hypotheses, the theoretical implications of our results must be interpreted with caution.

Nevertheless, our findings make a valuable contribution to the existing literature, as this is the first study to our knowledge to utilise the ANT in the study of acute stress. Furthermore, we have highlighted the importance of methodology in revealing the effects of stress on attentional processing, as both the detrimental and beneficial effects appear to be dependent on task demands.

References

Alilović, J., Timmermans, B., Reteig, L., van Gaal, S., & Slagter, H. (in press). No evidence that predictions and attention modulate the first feedforward sweep of cortical information processing. *bioRxiv.* doi:10.1101/351965

Allen, J. P., Litten, R. Z., Fertig, J. B., & Babor, T. (1997). A review of research on the Alcohol Use Disorders Identification Test (AUDIT). *Alcoholism: Clinical and Experimental Research, 21*(4), 613-619. doi:10.1111/j.1530-0277.1997.tb03811.x

Allman, J. M., Hakeem, A., Erwin, J. M., Nimchinsky, E., & Hof, P. (2001). The anterior cingulate cortex - The evolution of an interface between emotion and cognition. *Annals of the New York Academy of Sciences, 935*, 107-117. doi:10.1111/j.1749-6632.2001.tb03476.x

Andrews, G., & Slade, T. (2001). Interpreting scores on the Kessler Psychological Distress Scale (K10). *Australian and New Zealand Journal of Public Health, 25*(6), 494-497. doi:10.1111/j.1467-842X.2001.tb00310.x

Arnsten, A. F. (2009). Stress signalling pathways that impair prefrontal cortex structure and function. *Nature Reviews Neuroscience, 10*(6), 410-422. doi:10.1038/nrn2648

Arnsten, A. F. (2015). Stress weakens prefrontal networks: molecular insults to higher cognition. *Nature Neuroscience, 18*(10), 1376-1385. doi:10.1038/nn.4087

Aston-Jones, G., & Cohen, J. D. (2005a). Adaptive gain and the role of the locus coeruleus-norepinephrine system in optimal performance. *Journal of Comparative Neurology, 493*(1), 99-110. doi:10.1002/cne.20723

Aston-Jones, G., & Cohen, J. D. (2005b). An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. *Annual Review of Neuroscience, 28,* 403-450. doi:10.1146/annurev.neuro.28.061604.135709

Aston-Jones, G., Rajkowski, J., Kubiak, P., & Alexinsky, T. (1994). Locus coeruleus neurons in monkey are selectively activated by attended cues in a vigilance task. *Journal of Neuroscience, 14*(7), 4467-4480. doi:10.1523/JNEUROSCI.14-07-04467.1994

Babor, T. F., Higgins-Biddle, J. C., Saunders, J. B., & Monteiro, M. G. (2001). *The Alcohol Use Disorders Identification Test: Guidelines for use in primary care*. Retrieved from http://apps.who.int/iris/handle/10665/67205:

Callejas, A., Lupianez, J., Funes, M. J., & Tudela, P. (2005). Modulations among the alerting, orienting and executive control networks. *Experimental Brain Research, 167*(1), 27-37. doi:10.1007/s00221-005-2365-z

Carter, C. S., & van Veen, V. (2007). Anterior cingulate cortex and conflict detection: an update of theory and data. *Cognitive, Affective, and Behavioral Neuroscience, 7*(4), 367-379. doi:10.3758/CABN.7.4.367

Childs, E., Dlugos, A., & De Wit, H. (2010). Cardiovascular, hormonal, and emotional responses to the TSST in relation to sex and menstrual cycle phase. *Psychophysiology, 47*(3), 550-559. doi:10.1111/j.1469-8986.2009.00961.x

Cohen, J. D. (1988). *Statistical Power Analysis for the Behavioral Sciences* (2nd ed.). Hillsdale NJ:Erlbaum.

Dienes, Z. (2014). Using Bayes to get the most out of non-significant results. *Frontiers in Psychology, 5*. doi:10.3389/fpsyg.2014.00781

Dierolf, A. M., Fechtner, J., Bohnke, R., Wolf, O. T., & Naumann, E. (2017). Influence of acute stress on response inhibition in healthy men: An ERP study. *Psychophysiology, 54*(5), 684-695. doi:10.1111/psyp.12826

Elling, L., Steinberg, C., Brockelmann, A. K., Dobel, C., Bolte, J., & Junghofer, M. (2011). Acute stress alters auditory selective attention in humans independent of HPA: a study of evoked potentials. *PLoS One, 6*(4), e18009. doi:10.1371/journal.pone.0018009

Ermutlu, M. N., Karamursel, S., Ugur, E. H., Senturk, L., & Gokhan, N. (2005). Effects of cold stress on early and late stimulus gating. *Psychiatry Research, 136*(2-3), 201-209. doi:10.1016/j.psychres.2003.03.002

Everly, G. S., & Lasting, J. M. (2013) *A clinical guide to the treatment of the human stress response*. New York: Springer.

Fan, J., McCandliss, B. D., Fossella, J., Flombaum, J. I., & Posner, M. I. (2005). The activation of attentional networks. *Neuroimage, 26*(2), 471-479. doi:10.1016/j.neuroimage.2005.02.004

Fan, J., McCandliss, B. D., Sommer, T., Raz, A., & Posner, M. I. (2002). Testing the efficiency and independence of attentional networks. *Journal of Cognitive Neuroscience, 14*(3), 340-347. doi:10.1162/089892902317361886

Fan, J., & Posner, M. (2004). Human attentional networks. *Psychiatr Prax, 31 Suppl 2*, S210-214. doi:10.1055/s-2004-828484

Gagnon, S. A., & Wagner, A. D. (2016). Acute stress and episodic memory retrieval: neurobiological mechanisms and behavioral consequences. *Annals of the New York Academy of Sciences, 1369*(1), 55-75. doi:10.1111/nyas.12996

Galvao-Carmona, A., Gonzalez-Rosa, J. J., Hidalgo-Munoz, A. R., Paramo, D., Benitez, M. L., Izquierdo, G., & Vazquez-Marrufo, M. (2014). Disentangling the attention network test: behavioral, event related potentials, and neural source analyses. *Frontiers in Human Neuroscience, 8*, 813. doi:10.3389/fnhum.2014.00813

Gogos, A., Wu, Y. C., Williams, A. S., & Byrne, L. K. (2014). The Effects of Ethinylestradiol and Progestins ("the pill") on Cognitive Function in Pre-menopausal Women. *Neurochemical Research, 39*(12), 2288-2300. doi:10.1007/s11064-014-1444-6

Groom, M. J., & Cragg, L. (2015). Differential modulation of the N2 and P3 event-related potentials by response conflict and inhibition. *Brain and Cognition, 97*, 1-9. doi:10.1016/j.bandc.2015.04.004

Herman, J. P., McKlveen, J. M., Ghosal, S., Kopp, B., Wulsin, A., Makinson, R., . . . Myers, B. (2016). Regulation of the Hypothalamic-Pituitary-Adrenocortical Stress Response. *Comprehensive Physiology, 6*(2), 603-621. doi:10.1002/cphy.c150015

Hermans, E. J., van Marle, H. J., Ossewaarde, L., Henckens, M. J., Qin, S., van Kesteren, M. T., . . . Fernandez, G. (2011). Stress-related noradrenergic activity prompts large-scale neural network reconfiguration. *Science, 334*(6059), 1151-1153. doi:10.1126/science.1209603

Hillyard, S. A., Hink, R. F., Schwent, V. L., & Picton, T. W. (1973). Electrical signs of selective attention in the human brain. *Science, 182*(4108), 177-180. doi:10.1126/science.182.4108.177

Hillyard, S. A., Vogel, E. K., & Luck, S. J. (1998). Sensory gain control (amplification) as a mechanism of selective attention: electrophysiological and neuroimaging

evidence. *Philosophical Transactions of the Royal Society B, 353*(1373), 1257-1270. doi:10.1098/rstb.1998.0281

Hopf, J. M., & Mangun, G. R. (2000). Shifting visual attention in space: an electrophysiological analysis using high spatial resolution mapping. *Clinical Neurophysiology, 111*(7), 1241-1257. doi:10.1016/S1388-2457(00)00313-8

Howe, W. M., Ji, J., Parikh, V., Williams, S., Mocaer, E., Trocme-Thibierge, C., & Sarter, M. (2010). Enhancement of attentional performance by selective stimulation of alpha4beta2(*) nAChRs: underlying cholinergic mechanisms. *Neuropsychopharmacology, 35*(6), 1391-1401. doi:10.1038/npp.2010.9

Iacobucci, D., Posavac, S. S., Kardes, F. R., Schneider, M. J., & Popovich, D. L. (2015). Toward a more nuanced understanding of the statistical properties of a median split. *Journal of Consumer Psychology, 25*(4), 652-665. doi:10.1016/j.jcps.2014.12.002

Imperato, A., Puglisi-Allegra, S., Casolini, P., & Angelucci, L. (1991). Changes in brain dopamine and acetylcholine release during and following stress are independent of the pituitary-adrenocortical axis. *Brain Research, 538*(1), 111-117. doi:10.1016/0006-8993(91)90384-8

Jasper, H. H. (1958). Report of committee on methods of clinical examination in electroencephalography. *Electroencephalography and Clinical Neurophysiology, 10*(2), 370-375. doi:10.1016/0013-4694(58)90053-1

Jiang, C., Buchanan, T. W., Yao, Z., Zhang, K., Wu, J., & Zhang, L. (2017). Acute Psychological Stress Disrupts Attentional Bias to Threat-Related Stimuli. *Scientific Reports, 7*(1), 14607. doi:10.1038/s41598-017-14138-w

Jiang, C., & Rau, P. P. (2017). Working memory performance impaired after exposure to acute social stress: The evidence comes from ERPs. *Neuroscience Letters, 658*, 137-141. doi:10.1016/j.neulet.2017.08.054

Joels, M., Sarabdjitsingh, R. A., & Karst, H. (2012). Unraveling the time domains of corticosteroid hormone influences on brain activity: rapid, slow, and chronic modes. *Pharmacological Reviews, 64*(4), 901-938. doi:10.1124/pr.112.005892

Jonkman, L. M., Lansbergen, M., & Stauder, J. E. (2003). Developmental differences in behavioral and event-related brain responses associated with response preparation and inhibition in a go/nogo task. *Psychophysiology, 40*(5), 752-761. doi:10.1111/1469-8986.00075

Kessler, R. C., Andrews, G., Colpe, L. J., Hiripi, E., Mroczek, D. K., Normand, S. L., . . . Zaslavsky, A. M. (2002). Short screening scales to monitor population prevalences and trends in non-specific psychological distress. *Psychological Medicine, 32*(6), 959-976. doi:10.1017/S0033291702006074

Kim, D., Bae, H., & Park, Y. C. (2008). Validity of the Subjective units of Disturbance Scale in EMDR. *Journal of EDMR Practice and Research, 2*(1), 57-62. doi:10.1891/1933-3196.2.1.57

Kirschbaum, C., Kudielka, B. M., Gaab, J., Schommer, N. C., & Hellhammer, D. H. (1999). Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. *Psychosomatic Medicine, 61*(2), 154-162. doi:Doi 10.1097/00006842-199903000-00006

Kopp, B., Rist, F., & Mattler, U. (1996). N200 in the flanker task as a neurobehavioral tool for investigating executive control. *Psychophysiology, 33*(3), 282-294. doi:DOI 10.1111/j.1469-8986.1996.tb00425.x

Lange, K., & Roder, B. (2006). Orienting attention to points in time improves stimulus processing both within and across modalities. *Journal of Cognitive Neuroscience, 18*(5), 715-729. doi:10.1162/jocn.2006.18.5.715

Larra, M. F., Pramme, L., Schachinger, H., & Frings, C. (2016). Stress and selective attention: Immediate and delayed stress effects on inhibition of return. *Brain Cogn, 108*, 66-72. doi:10.1016/j.bandc.2016.07.008

Luck, S. J. (2014). *An introduction to the event-related potential technique* (2nd ed.). London, England: The MIT Press.

Luck, S. J., Woodman, G. F., & Vogel, E. K. (2000). Event-related potential studies of attention. *Trends in Cognitive Sciences, 4*(11), 432-440. doi:10.1016/S1364-6613(00)01545-X

Lupien, S. J., Maheu, F., Tu, M., Fiocco, A., & Schramek, T. E. (2007). The effects of stress and stress hormones on human cognition: Implications for the field of brain and cognition. *Brain and Cognition, 65*(3), 209-237. doi:10.1016/j.bandc.2007.02.007

Lupien, S. J., Wilkinson, C. W., Briere, S., Menard, C., Ng Ying Kin, N. M., & Nair, N. P. (2002). The modulatory effects of corticosteroids on cognition: studies in young human populations. *Psychoneuroendocrinology, 27*(3), 401-416. doi:10.1016/S0306-4530(01)00061-0

Matuszewich, L., McFadden, L. M., Friedman, R. D., & Frye, C. A. (2014). Neurochemical and behavioral effects of chronic unpredictable stress. *Behavioural Pharmacology, 25*(5-6), 557-566. doi:10.1097/FBP.0000000000000061

Nater, U. M., & Rohleder, N. (2009). Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: Current state of research. *Psychoneuroendocrinology, 34*(4), 486-496. doi:10.1016/j.psyneuen.2009.01.014

Neuhaus, A. H., Urbanek, C., Opgen-Rhein, C., Hahn, E., Ta, T. M., Koehler, S., . . . Dettling, M. (2010). Event-related potentials associated with Attention Network Test. *International Journal of Psychophysiology, 76*(2), 72-79. doi:10.1016/j.ijpsycho.2010.02.005

Nieoullon, A. (2002). Dopamine and the regulation of cognition and attention. *Progress in Neurobiology, 67*(1), 53-83. doi:10.1016/S0301-0082(02)00011-4

Nieuwenhuis, S., Aston-Jones, G., & Cohen, J. D. (2005). Decision making, the P3, and the locus coeruleus-norepinephrine system. *Psychological Bulletin, 131*(4), 510-532. doi:10.1037/0033-2909.131.4.510

Parikh, V., Kozak, R., Martinez, V., & Sarter, M. (2007). Prefrontal acetylcholine release controls cue detection on multiple timescales. *Neuron, 56*(1), 141-154. doi:10.1016/j.neuron.2007.08.025

Petersen, S. E., & Posner, M. I. (2012). The attention system of the human brain: 20 years after. *Annual Review of Neuroscience, 35*, 73-89. doi:10.1146/annurev-neuro-062111-150525

Picciotto, M. R., Higley, M. J., & Mineur, Y. S. (2012). Acetylcholine as a neuromodulator: cholinergic signaling shapes nervous system function and behavior. *Neuron, 76*(1), 116-129. doi:10.1016/j.neuron.2012.08.036

Pilgrim, K., Marin, M. F., & Lupien, S. J. (2010). Attentional orienting toward social stress stimuli predicts increased cortisol responsivity to psychosocial stress

irrespective of the early socioeconomic status. *Psychoneuroendocrinology, 35*(4), 588-595. doi:10.1016/j.psyneuen.2009.09.015

Posner, M. I. (2008). Measuring alertness. *Annals of the New York Academy of Sciences, 1129*, 193-199. doi:10.1196/annals.1417.011

Posner, M. I., & Petersen, S. E. (1990). The attention system of the human brain. *Annual Review of Neuroscience, 13*, 25-42. doi:10.1146/annurev.ne.13.030190.000325

Qi, M., Gao, H., & Liu, G. (2017). Effect of acute psychological stress on response inhibition: An event-related potential study. *Behavioural Brain Research, 323*, 32-37. doi:10.1016/j.bbr.2017.01.036

Qi, M., Gao, H., & Liu, G. (2018). The effect of mild acute psychological stress on attention processing: an ERP study. *Experimental Brain Research, 236*(7), 2061-2071. doi:10.1007/s00221-018-5283-6

Raz, A. (2004). Anatomy of attentional networks. *Anatomical Record. Part B, New Anatomist, 281*(1), 21-36. doi:10.1002/ar.b.20035

Reinert, D. F., & Allen, J. P. (2007). The Alcohol Use Disorders Identification Test: An update of research findings. *Alcoholism-Clinical and Experimental Research, 31*(2), 185-199. doi:10.1111/j.1530-0277.2006.00295.x

Roca, J., Castro, C., Lopez-Ramon, M. F., & Lupianez, J. (2011). Measuring vigilance while assessing the functioning of the three attentional networks: The ANTI-Vigilance task. *Journal of Neuroscience Methods, 198*(2), 312-324. doi:10.1016/j.jneumeth.2011.04.014

Sanger, J., Bechtold, L., Schoofs, D., Blaszkewicz, M., & Wascher, E. (2014). The influence of acute stress on attention mechanisms and its electrophysiological

correlates. *Frontiers in Behavioral Neuroscience, 8,* 353. doi:10.3389/fnbeh.2014.00353

Sara, S. J. (2009). The locus coeruleus and noradrenergic modulation of cognition. *Nature Reviews Neuroscience, 10*(3), 211-223. doi:10.1038/nrn2573

Sato, H., Takenaka, I., & Kawahara, J. I. (2012). The effects of acute stress and perceptual load on distractor interference. *The Quarterly of Experimental Psychology, 65*(4), 617-623. doi:10.1080/17470218.2011.648944

Saunders, J. B., Aasland, O. G., Babor, T. F., de la Fuente, J. R., & Grant, M. (1993). Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO Collaborative Project on Early Detection of Persons with Harmful Alcohol Consumption--II. *Addiction, 88*(6), 791-804. doi:10.1111/j.1360-0443.1993.tb02093.x

Schmajuk, M., Liotti, M., Busse, L., & Woldorff, M. G. (2006). Electrophysiological activity underlying inhibitory control processes in normal adults. *Neuropsychologia, 44*(3), 384-395. doi:10.1016/j.neuropsychologia.2005.06.005

Schwabe, L., Haddad, L., & Schachinger, H. (2008). HPA axis activation by a socially evaluated cold-pressor test. *Psychoneuroendocrinology, 33*(6), 890-895. doi:10.1016/j.psyneuen.2008.03.001

Shackman, A. J., Maxwell, J. S., McMenamin, B. W., Greischar, L. L., & Davidson, R. J. (2011). Stress potentiates early and attenuates late stages of visual processing. *Journal of Neuroscience, 31*(3), 1156-1161. doi:10.1523/JNEUROSCI.3384-10.2011

Shields, G. S., Sazma, M. A., & Yonelinas, A. P. (2016). The effects of acute stress on core executive functions: A meta-analysis and comparison with cortisol.

Neuroscience and Biobehavioral Reviews, 68, 651-668. doi:10.1016/j.neubiorev.2016.06.038

Shilton, A. L., Laycock, R., & Crewther, S. G. (2017). The Maastricht Acute Stress Test (MAST): Physiological and Subjective Responses in Anticipation, and Post-stress. *Frontiers in Psychology, 8*, 567. doi:10.3389/fpsyg.2017.00567

Slade, T., Grove, R., & Burgess, P. (2011). Kessler Psychological Distress Scale: normative data from the 2007 Australian National Survey of Mental Health and Wellbeing. *Australian and New Zealand Journal of Psychiatry, 45*(4), 308-316. doi:10.3109/00048674.2010.543653

Smeets, T., Cornelisse, S., Quaedflieg, C. W., Meyer, T., Jelicic, M., & Merckelbach, H. (2012). Introducing the Maastricht Acute Stress Test (MAST): a quick and non-invasive approach to elicit robust autonomic and glucocorticoid stress responses. *Psychoneuroendocrinology, 37*(12), 1998-2008. doi:10.1016/j.psyneuen.2012.04.012

Tsigos, C., & Chrousos, G. P. (2002). Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. *Journal of Psychosomatic Research, 53*(4), 865-871. doi:10.1016/S0022-3999(02)00429-4

Ulrich-Lai, Y. M., & Herman, J. P. (2009). Neural regulation of endocrine and autonomic stress responses. *Nature Reviews Neuroscience, 10*(6), 397-409. doi:10.1038/nrn2647

van Marle, H. J. F., Hermans, E. J., Qin, S. Z., & Fernandez, G. (2009). From Specificity to Sensitivity: How Acute Stress Affects Amygdala Processing of Biologically Salient Stimuli. *Biological Psychiatry, 66*(7), 649-655. doi:10.1016/j.biopsych.2009.05.014

van Veen, V., Cohen, J. D., Botvinick, M. M., Stenger, V. A., & Carter, C. S. (2001). Anterior cingulate cortex, conflict monitoring, and levels of processing. *Neuroimage, 14*(6), 1302-1308. doi:10.1006/nimg.2001.0923

Vogel, S., Fernandez, G., Joels, M., & Schwabe, L. (2016). Cognitive Adaptation under Stress: A Case for the Mineralocorticoid Receptor. *Trends in Cognitive Sciences, 20*(3), 192-203. doi:10.1016/j.tics.2015.12.003

Wagenmakers, E. J., Love, J., Marsman, M., Jamil, T., Ly, A., Verhagen, J., . . . Morey, R. D. (2018). Bayesian inference for psychology. Part II: Example applications with JASP. *Psychonomic Bulletin & Review, 25*(1), 58-76. doi:10.3758/s13423-017-1323-7

Wand, G. S., Oswald, L. M., McCaul, M. E., Wong, D. F., Johnson, E., Zhou, Y., . . . Kumar, A. (2007). Association of amphetamine-induced striatal dopamine release and cortisol responses to psychological stress. *Neuropsychopharmacology, 32*(11), 2310-2320. doi:10.1038/sj.npp.1301373

Wang, H., & Fan, J. (2007). Human attentional networks: a connectionist model. *Journal of Cognitive Neuroscience, 19*(10), 1678-1689. doi:10.1162/jocn.2007.19.10.1678

Williams, R. S., Biel, A. L., Wegier, P., Lapp, L. K., Dyson, B. J., & Spaniol, J. (2016). Age differences in the Attention Network Test: Evidence from behavior and event-related potentials. *Brain and Cognition, 102*, 65-79. doi:10.1016/j.bandc.2015.12.007

Wolpe, J. (1969). *The practice of behaviour therapy*. New York: Pergamon Press.

Woodman, G. F. (2010). A brief introduction to the use of event-related potentials in studies of perception and attention. *Attention, Perception, and Psychophysics, 72*(8), 2031-2046. doi:10.3758/APP.72.8.2031

Zimmer, C., Basler, H. D., Vedder, H., & Lautenbacher, S. (2003). Sex differences in cortisol response to noxious stress. *The Clinical Journal of Pain, 19*(4), 233-239. doi:10.1097/00002508-200307000-00006

Appendix A

Online Screening Questionnaire

1. How old are you? _____

2. What is your sex? **male** 1**/ female** 2**/ other** 3 *(please detail*)

_____

if female:

are you currently breastfeeding **yes / no**

is there any possibility that you could be pregnant **yes / no**

What was the date of the first day of your most recent period? If you don't remember the exact date give an approximate range (e.g. 5-8 May): _____

3. Is English the only language you have ever spoken fluently? **yes / no**

4. Are you right or left handed? **right** 1**/ left** 2

5. Do you have particularly sensitive skin? **yes / no**

Skin preparation for EEG recording includes the use of alcohol wipes and exfoliant in order to get the best reading from electrodes, people with sensitive skin may find this irritating.

6. Have you ever had or are you now suffering from any of the following:

Fits or convulsions	**yes**	**no**
Epilepsy	**yes**	**no**
Giddiness	**yes**	**no**
Concussion	**yes**	**no**
Severe head injury	**yes**	**no**
Loss of consciousness	**yes**	**no**

Diabetes **yes** **no**

7. Do you have a heart condition or any other serious physical or neurological condition (including visual agnosia)? **yes / no**

if yes: please detail

_____

_____

_____

8. Are you currently suffering from anxiety? **yes / no**

9. Are you currently suffering from depression? **yes / no**

10. Do you have any other serious mental health condition? **yes / no**

if yes: please detail

_____

_____

_____

11. Have you suffered from anxiety, depression or any other mental health condition in the past? **yes / no**

if yes: please detail

_____

_____

_____

12. Are you currently taking any prescribed medications? **yes / no**

if yes: please detail

_____

_____

_____

13. Have you been prescribed medications in the past for mental health problems?

yes /no

if yes: please detail

_____

_____

_____

14. Do you have any difficulties with vision? **yes / no**

if yes: please specify

_____

_____

if yes: are these difficulties corrected (i.e. with glasses or contacts)? **yes / no**

15. Do you have any known difficulties with hearing? **yes / no**

if yes: please specify

_____

_____

The following questions are about your use of tobacco, alcohol and other substances

16. How often have you smoked tobacco in the last 6 months? (*Please circle*)

never	0
less than monthly	1
monthly	2
weekly	3

daily or almost daily 4

17. How often do you have a drink containing alcohol? (*Please circle*)

never 0

monthly or less 1

2-4 times per month 2

2-3 times per week 3

4 or more times per week 4

18. How many drinks containing alcohol do you have on a typical day when you are drinking?

1 or 2 0

3 or 4 1

5 or 6 2

7 to 9 3

10 or more 4

19. How often do you have six or more drinks on one occasion?

never 0

less than monthly 1

monthly 2

weekly 3

daily or almost daily 4

20. In the last 6 months, how often have you used illicit drugs (e.g., cannabis, ecstasy, speed)?

never 0

less than monthly 1

monthly	2
weekly	3
daily or almost daily	4

21. On how many occasions have you <u>ever</u> used illicit drugs?

None	0
1-5	1
5-10	2
10-15	3
More than 15 occasions	4

22. Do you have a sleep disorder or any sleeping difficulties? **yes / no**

*if yes, please detail*_____

_____

23. On average, how many hours do you sleep on a

weeknight:_____

weekend _____

24. Do you work night shifts or double shifts? **yes / no**

if yes, how many times per week?

_____

25. Are you taking hormonal contraceptives (females only)? **yes/no**

i*f yes, what type?*

_____

26. Is there any medical reason why you may not have your blood taken (males only)?

y**es/no**

if yes, why?

_____

27. Do you, or have you ever, had any blood-born diseases (eg. Hep B, Hep C, HIV, etc)?

yes/no

Kessler Psychological Distress scale (K10)

In the last 4 weeks, about how often:
1. Did you feel tired out for no good reason?
All of the time.....1
Most of the time.....2
Some of the time.....3
A little of the time.....4
None of the time 5

2. Did you feel nervous?
All of the time.....1
Most of the time.....2
Some of the time.....3
A little of the time.....4
None of the time 5
Note: If response 5 chosen, go to Q4
3. Did you feel so nervous that nothing could calm you down?
All of the time.....1
Most of the time.....2
Some of the time.....3
A little of the time.....4
None of the time 5

4. Did you feel hopeless?
All of the time.....1
Most of the time.....2
Some of the time.....3
A little of the time.....4
None of the time 5

5. Did you feel restless or fidgety?
All of the time.....1
Most of the time.....2
Some of the time.....3

A little of the time.....4
None of the time 5
Note: If response 5 chosen, go to Q7

6. Did you feel so restless that you could not sit still?
All of the time.....1
Most of the time.....2
Some of the time.....3
A little of the time.....4
None of the time.....5
7. Did you feel depressed?
All of the time.....1
Most of the time.....2
Some of the time.....3
A little of the time.....4
None of the time.....5

8. Did you feel that everything was an effort?
All of the time.....1
Most of the time.....2
Some of the time.....3
A little of the time.....4
None of the time 5

9. Did you feel so sad that nothing could cheer you up?
All of the time.....1
Most of the time.....2
Some of the time.....3
A little of the time.....4
None of the time 5

10. Did you feel worthless?
All of the time.....1
Most of the time.....2
Some of the time.....3
A little of the time.....4
None of the time 5

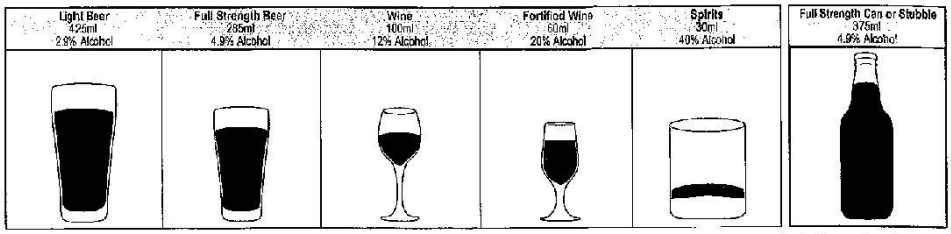
Alcohol Use Disorders Identification Test (AUDIT)

Australian Government
Department of Veterans' Affairs

ID: Mega_____

Alcohol Screen (AUDIT)

THE RIGHT MIX



*The guide above contains examples of **one standard drink**.*

A full strength can or stubbie contains ***one and a half standard drinks.***

Introduction

Because alcohol use can affect health and interfere with certain medications and treatments, it is important that we ask you some questions about your use of alcohol. Your answers will remain confidential, so please be as accurate as possible. Try to answer the questions in terms of '**standard drinks**'. Please ask for clarification if required.

AUDIT Questions Please tick the response that best fits your drinking.

						Score	Sub totals
	Never	*Monthly or less*	*2 - 4 times a month*	*2 - 3 times a week*	*4 or more times a week*		
1. How often do you have a drink containing alcohol?	☐ Go to Qs 9 & 10	☐	☐	☐	☐	☐	
	1 or 2	*3 or 4*	*5 or 6*	*7 to 9*	*10 or more*		
2. How many standard drinks do you have on a typical day when you are drinking?	☐	☐	☐	☐	☐	☐	
	Never	*Less than monthly*	*Monthly*	*Weekly*	*Daily or almost daily*		
3. How often do you have six or more standard drinks on one occasion ?	☐	☐	☐	☐	☐	☐	☐
4. How often during the last year have you found that you were not able to stop drinking once you had started?	☐	☐	☐	☐	☐	☐	
5. How often during the last year have you failed to do what was normally expected of you because of drinking?	☐	☐	☐	☐	☐	☐	
6. How often during the last year have you needed a first drink in the morning to get yourself going after a heavy drinking session?	☐	☐	☐	☐	☐	☐	☐
7. How often during the last year have you had a feeling of guilt or remorse after drinking?	☐	☐	☐	☐	☐	☐	
8. How often during the last year have you been unable to remember what happened the night before because you had been drinking?	☐	☐	☐	☐	☐	☐	
	No		*Yes, but not in the last year*		*Yes, during the last year*		
9. Have you or someone else been injured because of your drinking?	☐		☐		☐	☐	
10. Has a relative, friend, doctor, or other health care worker been concerned about your drinking or suggested you cut down?	☐		☐		☐	☐	☐
						TOTAL	

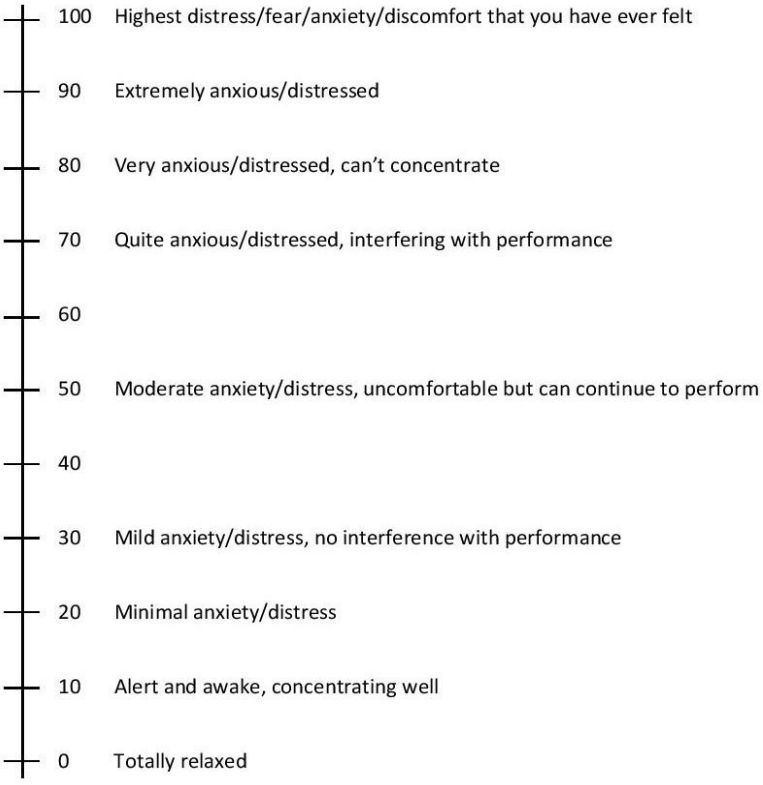
Supplementary Questions	*No*	*Probably Not*	*Unsure*	*Possibly*	*Definitely*
Do you think you presently have a problem with drinking?	☐	☐	☐	☐	☐
	Very easy	*Fairly easy*	*Neither difficult nor easy*	*Fairly difficult*	*Very difficult*
In the next 3 months, how difficult would you find it to cut down or stop drinking?	☐	☐	☐	☐	☐

Appendix B

Subjective Units of Distress Scale (SUDS)

The distress thermometer – Subjective Units of Distress Scale (SUDS)

Try to get used to rating your distress, fear, anxiety or discomfort on a scale of 0-100. Imagine you have a 'distress thermometer' to measure your feelings according to the following scale. Notice how your level of distress and fear changes over time and in different situations.



Appendix C

Ethics Approval Letter

Office of Research Services
University of Tasmania
Private Bag 1
Hobart Tasmania 7001
Telephone + 61 3 6226 7479
Facsimile + 61 3 6226 7148
Email Human.Ethics@utas.edu.au
www.research.utas.edu.au/human_ethics/

HUMAN RESEARCH ETHICS COMMITTEE (TASMANIA) NETWORK

UTAS

04 December 2017

Dr Allison Matthews
C/- University of Tasmania

Sent via email

Dear Dr Matthews

REF NO: H0016793

TITLE: A Translational Investigation of the Influence of Ovarian Hormones on Endocannabinoid-Modulated Negative Feedback of the HPA Stress Response

Document	***Version***	***Date***
HREA Application	Version 1	18 Oct 2017
Ethics Protocol dated 21 Aug 2017	Version 1	18 Oct 2017
Finance and Administration form		

The Tasmanian Health and Medical Human Research Ethics Committee considered and approved the above documentation on **28 November 2017** to be conducted at the following site(s):

University of Tasmania - Psychology Research Lab (Cognitive Neuro)

Please ensure that all investigators involved with this project have cited the approved versions of the documents listed within this letter and use only these versions in conducting this research project.

This approval constitutes ethical clearance by the Health and Medical HREC. The decision and authority to commence the associated research may be dependent on factors beyond the remit of the ethics review process. For example, your research may need ethics clearance from other organisations or review by your research governance coordinator or Head of Department. It is your responsibility to find out if the approvals of other bodies or authorities are required. It is recommended that the proposed research should not commence until you have

satisfied these requirements.

All committees operating under the Human Research Ethics Committee (Tasmania) Network are registered and required to comply with the *National Statement on the Ethical Conduct in Human Research* (NHMRC 2007 updated 2014).

Therefore, the Chief Investigator's responsibility is to ensure that:

1) The individual researcher's protocol complies with the HREC approved protocol.
2) Modifications to the protocol do not proceed until **approval** is obtained in writing from the HREC. Please note that all requests for changes to approved documents must include a version number and date when submitted for review by the HREC.
3) Section 5.5.3 of the National Statement states:

> Researchers have a significant responsibility in monitoring approved research as they are in the best position to observe any adverse events or unexpected outcomes. They should report such events or outcomes promptly to the relevant institution/s and ethical review body/ies and take prompt steps to deal with any unexpected risks.
>
> The appropriate forms for reporting such events in relation to clinical and non-clinical trials and innovations can be located at the website below. All adverse events must be reported regardless of whether or not the event, in your opinion, is a direct effect of the therapeutic goods being tested. http://www.utas.edu.au/research-admin/research- integrity-and-ethics-unit-rieu/human-ethics/human-research-ethics-review- process/health-and-medical-hrec/managing-your-approved-project

4) All research participants must be provided with the current Patient Information Sheet and Consent Form, unless otherwise approved by the Committee.
5) The Committee is notified if any investigators are added to, or cease involvement with, the project.
6) This study has approval for four years contingent upon annual review. A *Progress Report* is to be provided on the anniversary date of your approval. Your first report is due 28 November 2018**.** You will be sent a courtesy reminder closer to this due date.
7) A *Final Report* and a copy of the published material, either in full or abstract, must be provided at the end of the project.

Should you have any queries please do not hesitate to contact me on (03) 6226 6254.
Yours sincerely

Jude Vienna-Hallam
Ethics Administration Office

Appendix D

Participant Information Sheet

Study title: A Translational Investigation of Endocannabinoid-Modulated Negative Feedback of the HPA Stress Response: Pilot Study

Student Investigators

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Chief Investigator:

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Associate Investigators:

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Before you decide whether to participate in this study, it is important to understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with the researchers if you wish.

What is the purpose of this study?

The purpose of this study is to investigate the effect of a stressful task on the biological stress response system. Measurement of hormones (endocannabinoids and cortisol) in your saliva samples will tell us about you biological stress response following the task. This information will help us to understand the role of endocannabinoids in response to stress, which may later enable us to develop treatments that will help people with stress-related illnesses. We are also interested in the effects of acute stress on brain activity while people complete attention and memory tasks.

Why have I been invited to enter the study?

You have been invited to participate because you are enrolled as an undergraduate Psychology student at the University of Tasmania, or are otherwise interested in participating, and have reported that you do not suffer from psychiatric, neurological or cardiac illness, take drugs or currently use medication.

What does the study involve?

If you agree to participate, you will be screened for eligibility via an online questionnaire (you will need to be aged 18-40, not pregnant, not on current medications, using illicit drugs, use alcohol excessively or have a current psychiatric diagnosis, cardiac condition or neurological condition or epilepsy, or have previously undergone the Maastricht Acute Stress Test) and asked to sign the Participant Consent Form. You will then come in for an assessment (which will take between 2 and 3 hours) at the Cognitive Neuroscience Laboratory, UTAS. In this session, you will complete the following tasks.

Stress Task: In this task, you will alternate between putting you hand in a bucket of water and completing some mental arithmetic tasks. The water will either be at room temperature or it may be very cold (0-2 degrees). During this task, your face may be video recorded so we can later examine your facial expressions (but we will check whether this is ok with you first). There is no physical risk involved in this task. You are able to withdraw your hand from the water at any point and may still continue with the experiment.

Saliva samples: You will be asked to provide saliva samples on several occasions by placing saliva into a small plastic tube. This sample will be de-identified and given an ID number, it will be analysed for the stress hormone cortisol, endocannabinoids (anandamide and 2-AG) and gonadal hormones (oestrogen and progesterone) at the pathology labs in the School of Medicine, UTAS. Following processing, the saliva samples will be immediately destroyed.

Hair sample: You will be asked to provide a hair sample, which will be cut off from the base of your head by the experimenter. The hair taken will be from the back of the head, and approximately a pencil-width of hair will be taken at one time point. This sample will be de-identified and given an ID number, it will be analysed for endocannabinoids (anandamide and 2-AG) and gonadal hormones (oestrogen and progesterone) at the pathology labs in the School of Medicine, UTAS. Following processing, the sample will be destroyed.

Mood and Stress Questionnaires: You will complete questionnaires about your mood and level of stress at several time points during the session.

Attention and Memory Tasks: You will be asked to complete some attention and memory tasks on a computer. During these tasks, you will be asked to respond as quickly and as accurately as possible to particular objects which appear on the screen.

Measurement of brain activity: You will be fitted with a cap, which will measure your brain activity during the attention and memory tasks. This will involve a 30 minute setup where gel will be applied to the cap. Afterwards, you may have gel in your hair, but it washes out easily and there is no risk of physical danger during this process.

Are there any risks?

Some participants may feel uncomfortable answering questionnaires about psychiatric history and current mood and stress. It should be stressed that all responses and task performance levels are anonymous and kept strictly confidential and you cannot be identified by your data. Also, you are not obligated to answer any questions that cause discomfort, and you can withdraw from the study at any stage without penalty.

The equipment used to measure brain activity may feel a little uncomfortable, however it is not painful and there are no specific risks associated with measuring brain activity. If you have sensitive skin, there is a small possibility that you may have a slight skin reaction from the electrode preparation materials. You are advised to reconsider participation if you believe your skin may react.

If you do feel upset or uncomfortable at any point please let the researcher know. If you wish, a referral may be given to receive assessment or intervention in relation to previous trauma or

psychiatric conditions at a free psychological treatment service (The University Psychology Clinic, Ph: 62262805, email: clinic@psychol.utas.edu.au). Further, you may also attend the University counselling service by phoning 1800 817 675 to arrange a confidential appointment.

Some individuals may feel slightly embarrassed by providing saliva samples. You can provide this sample behind a screen or in the bathroom if you request it.

Are there any benefits?

We cannot guarantee that you will receive any benefits from this study. You may learn more about psychological research and you may gain further knowledge about biological responses following acute stress after receiving group results from the study.

What happens if I don't want to take part in the study?

Participation in this study is voluntary. It is completely up to you whether or not you participate. If you decide not to participate, it will not affect any current or future studies, or your relationship with the University of Tasmania. If you wish to withdraw from the study once it has started, you can do so at any time without having to give a reason and without penalty.

How will my confidentiality be protected?

All aspects of this study, including results will be strictly confidential and only the researchers will have access to your personal information. Confidentiality will be maintained at all times and information will not be made available to participants or others outside the study. All data will be de-identified and stored using an ID number only. All data will be stored in a locked office, or in a computer database that will be password protected.

What happens with the results?

If you give us your permission by signing the consent document, we plan to discuss the results among at conferences and other forums, and publish the results in scientific journals. Any publication of results will only use de-identified information, and data is not analysed individually, but only as part of larger group analyses.

Will taking part in this study cost me anything, and will I be paid?

Participation in this study will not cost you anything. In addition, you will choose to either receive 3 hours course credit for your psychology 1 studies, or a $30 gift voucher to cover your expenses.

How is this study being paid for?

This study is being sponsored by the National Health and Medical Research Council (NHMRC). No commercial bodies have an interest in this research project.

Who should I contact if I have concerns about the conduct of this study?

If you would like to discuss any aspect of this study, please feel free to contact Dr Allison Matthews on ph (03) 62267236 or email Allison.Matthews@utas.edu.au

This study has been approved by the Tasmanian Health and Medical Human Research Ethics Committee. Any person with concerns or complaints about the conduct of this study should contact the Research Office nominated to receive complaints from research participants on 03 6226 7479 and quote [HREC project No H0016793]

Thank you for taking the time to consider this study.

If you wish to take part in it, please sign the attached consent form.

This information sheet is for you to keep.

Appendix E

Consent Form

Study title: A Translational Investigation of the Influence of Ovarian Hormones on Endocannabinoid-Modulated Negative Feedback of the HPA Stress Response

1. I,.....
   agree to participate in the study described in the information sheet above.
2. I acknowledge that I have read the participant information statement, which explains why I have been selected, the aims of the study and the nature and the possible risks of the investigation, and the statement has been explained to me to my satisfaction.
3. Before signing this consent form, I have been given the opportunity to ask any questions relating to any possible physical and mental harm and I have received satisfactory answers.
4. I understand that I can withdraw from the study at any time without prejudice to my relationship to University of Tasmania.
5. I agree that research data gathered from the results of the study may be published, provided that I cannot be identified.
6. I understand and agree that hair and saliva samples will be taken during the study.
7. I understand that my facial expressions may be videotaped during the stress task (which involves performing mental arithmetic tasks and immersing my hand in warm or cold water).
8. I understand that if I have any questions relating to my participation in this research, I may contact Dr Allison Matthews on 62267236 or Allison.Matthews@utas.edu.au
9. I acknowledge that I have been given a copy of the Participant Information Statement to keep.

Signature of subject Please PRINT name Date

_____

Signature of investigator Please PRINT name Date

_____

Appendix F

Experimental Session Questionnaire

Date ____ /____ /____ ID Number_____

1. Have you abstained from illicit drugs since the screening interview*?* □ **Criteria fulfilled**

If criterion has not been fulfilled, do not record any information on this sheet

2. Have you consumed alcohol within the last 24 hours? **yes / no**

3. How many cups of coffee (or other caffeinated drinks/products) have you consumed today?

_____

if >0: how many hours since your last caffeinated drink?

_____

4. Have you had any tobacco or nicotine products today? **yes / no**

if yes: how many cigarettes/nicotine products have you had today?

_____

if yes: how many hours has it been since your last cigarette or nicotine product?

_____

5. Have you consumed any medications in the past week (or since the screening interview)? **yes / no**

if yes, please detail:

Medication	Number of occasions taken	Time since last taken	Estimated dose

Appendix G

Statistical Output for Non-theoretically Relevant Effects

Table G1.

Non-theoretically relevant F-tests for Reaction Time (ms).

Effect	*F*-test
Cue by Condition	$F(1.6, 57.5)=0.33$, $p=.677$, $\eta_p^2=.009$
Flanker Congruency by Condition	$F(1, 35)=0.11$, $p=.741$, $\eta_p^2=.003$
Cue by Flanker Congruency	$F(1.7, 58.6)=32.33$, $p<.000$, $\eta_p^2=.480$
Time by Flanker Congruency	$F(1, 35)=9.89$, $p=.003$, $\eta_p^2=.220$
Time by Cue	$F(1.6, 55.7)=3.58$, $p=.044$, $\eta_p^2=.093$
Time by Condition	$F(1,35)=0.33$, $p=.856$, $\eta_p^2=.001$
Time by Cue by Condition	$F(1.6, 55.7)=0.11$, $p=.857$, $\eta_p^2=.003$
Time by Cue by Flanker Congruency	$F(1.9, 67.4)=2.00$, $p=.144$, $\eta_p^2=.054$
Cue by Flanker Congruency by Condition	$F(1.7, 58.6)=0.26$, $p=.731$, $\eta_p^2=.007$
Time by Cue by Flanker Congruency by Condition	$F(1.9, 67.4)=0.36$, $p=.697$, $\eta_p^2=.010$

Table G2.

Non-theoretically relevant F-tests for Accuracy (% Correct).

Effect	*F-test*
Cue by Condition	$F(1.8, 63.5)=2.53$, $p=.093$, $\eta_p^2=.067$
Flanker Congruency by Condition	$F(1, 35)=0.00$, $p=.981$, $\eta_p^2=.000$
Time by Cue	$F(1.9, 66.5)=1.82$, $p=.173$, $\eta_p^2=.049$
Time by Flanker Congruency	$F(1, 35)=0.03$, $p=.862$, $\eta_p^2=.001$
Time by Condition	$F(1, 35)=0.21$, $p=.653$, $\eta_p^2=.006$
Time by Cue by Condition	$F(1.9, 66.5)=1.28$, $p=.285$, $\eta_p^2=.035$
Cue by Flanker by Condition	$F(1.5, 67.4)=0.96$, $p=.366$, $\eta_p^2=.027$
Time by Cue by Flanker	$F(1.9, 66.6)=0.66$, $p=.512$, $\eta_p^2=.019$
Time by Cue by Congruency by Condition	$F(1.9, 66.6)=2.06$, $p=.138$, $\eta_p^2=.056$

Table G3.

Non-theoretically relevant F-tests for N1 amplitude (μV).

Effect	*F*-test
Flanker Congruency	$F(1, 35)=0.01, p=.907, \eta_p^2=.000$
Cue by Condition	$F(1.7, 57.7)=6.42, p=.529, \eta_p^2=.018$
Flanker Congruency by Condition	$F(1, 35)=5.24, p=.028, \eta_p^2=.130$
Time by Cue	$F(2.0, 68.2)=0.35, p=.708, \eta_p^2=.010$
Time by Flanker Congruency	$F(1, 35)=0.20, p=.656, \eta_p^2=.006$
Cue by Flanker Congruency	$F(1.8, 63.1)=3.93, p=.024, \eta_p^2=.101$
Time by Flanker Congruency by Condition	$F(1, 35)=0.49, p=.489, \eta_p^2=.014$
Cue by Flanker Congruency by Condition	$F(1.8, 63.1)=0.12, p=.885, \eta_p^2=.003$
Time by Cue by Flanker Congruency	$F(2.0, 69.1)=0.07, p=.929, \eta_p^2=.002$
Time by Cue by Flanker Congruency by Condition	$F(2.0, 69.1)=1.95, p=.149, \eta_p^2=.052$

Table G4.

Non-theoretically relevant F-tests for P3 amplitude (μV).

Effect	*F*-test
Cue	$F(1.4, 48.8)=51.63$, $p<.001$, $\eta_p^2=.596$
Time by Condition	$F(1, 35)=0.21$, $p=.651$, $\eta_p^2=.006$
Cue by Condition	$F(1.4, 48.8)=0.87$, $p=.389$, $\eta_p^2=.024$
Flanker Congruency by Condition	$F(1, 35)=0.026$, $p=.872$, $\eta_p^2=.001$,
Time by Flanker Congruency	$F(1, 35)=0.00$, $p=.974$, $\eta_p^2=.000$.
Time by Cue	$F(2.0, 66.8)=10.81$, $p<.001$, $\eta_p^2=.236$
Cue by Flanker Congruency	$F(1.9, 66.6)=0.88$, $p=.417$, $\eta_p^2=.024$
Time by Cue by Condition	$F(2.0, 66.8)=0.20$, $p=.821$, $\eta_p^2=.006$
Cue by Congruency by Condition	$F(1.9, 66.7)=0.139$, $p=.861$, $\eta_p^2=.004$
Time by Cue by Flanker Congruency	$F(1.7, 60.1)=0.35$, $p=.675$, $\eta_p^2=.010$
Time by Cue by Flanker Congruency by Condition	$F(1.7, 60.1)=0.18$, $p=.802$, $\eta_p^2=.005$

Appendix H

Statistical Output for Supplementary Analyses

Table H1.

F-tests for Block analysis of participants' reaction time (ms) post-stress manipulation.

Effect	*F*-test
Cue	$F(1.5, 51.5)=106.40$, $p<.001$, $\eta_p^2=.752$
Flanker	$F(1, 35)=389.93$, $p<.001$, $\eta_p^2=.918$
Block	$F(1.9, 65.7)=35.47$, $p<.001$, $\eta_p^2=.503$
Condition	$F(1, 35)=0.24$, $p=.629$, $\eta_p^2=.007$
Cue by Condition	$F(1.5, 51.5)=0.47$, $p=.571$, $\eta_p^2=.013$
Flanker by Condition	$F(1, 35)=0.20$, $p=.655$, $\eta_p^2=.006$
Block by Condition	$F(1.89, 65.7)=1.43$, $p=.238$, $\eta_p^2=.039$
Cue by Flanker	$F(1.23, 44.9)=8.92$, $p<.001$, $\eta_p^2=.203$
Cue by Block	$F(2.3, 79.9)=2.42$, $p=.088$, $\eta_p^2=.065$
Flanker by Block	$F(1.56, 55.0)=7.14$, $p=.004$, $\eta_p^2=.169$
Cue by Condition by Block	$F(2.3, 79.9)=2.14$, $p=.118$, $\eta_p^2=.058$
Cue by Flanker by Condition	$F(1.3, 44.9)=0.52$, $p=.520$, $\eta_p^2=.015$
Flanker by Condition by Block	$F(1.6, 55.0)=0.71$, $p=.465$, $\eta_p^2=.020$
Cue by Flanker by Block	$F(4.3, 151.5)=2.38$, $p=.049$, $\eta_p^2=.064$
Cue by Flanker by Block by Condition	$F(4.3, 151.5)=0.891$, $p=.477$, $\eta_p^2=.025$

Table H2.

Statistical output for supplementary regression analysis between cortisol reactivity (Time 3 – Baseline) and peak P3 amplitude for incongruent flankers post-stress.

Condition	*r*	R^2	*β*	*F (df)*	*p*	95% CI[*LL*, *UL*]
Placebo	-.062	.004	-0.55	0.06 (1,16)	.806	[-5.18, 4.09]
MAST	.209	.043	0.54	0.73 (1,16)	.406	[-0.80, 1.87]

Note. CI = confidence interval; *LL* = lower limit; *UL* = upper limit.

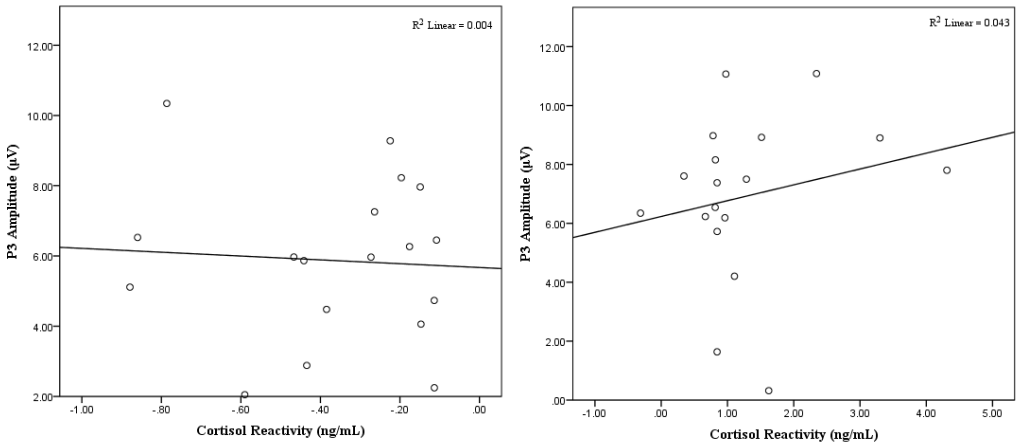

Figure H1. Scatter plots of cortisol reactivity (Time 3 – Baseline) versus P3 amplitude for incongruent flankers post-stress for the control (left) and stress (right) conditions.

Table H3.

Statistical output for supplementary regression analysis between cortisol reactivity (Time 3 – Baseline) and reaction time for incongruent flankers post-stress.

Condition	*r*	R^2	*β*	*F (df)*	*p*	95% CI[*LL*, *UL*]
Placebo	-.284	0.08	-51.67	1.40 (1,16)	.254	[-144.22, 40.89]
MAST	-.014	.000	-0.92	0.00 (1,16)	.957	[-36.43, 34.58]

Note. CI = confidence interval; *LL* = lower limit; *UL* = upper limit.

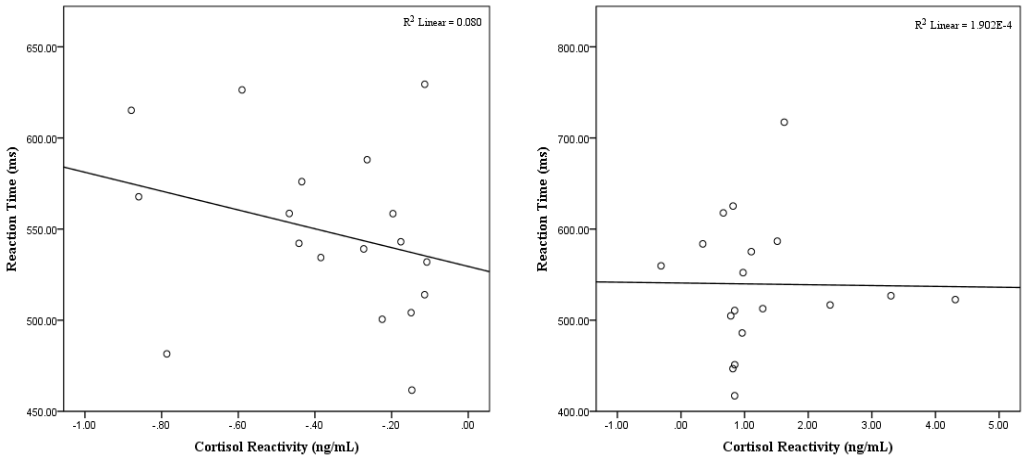

Figure H2. Scatter plots of cortisol reactivity (Time 3 – Baseline) versus reaction time for incongruent flankers post-stress for the control (left) and stress (right) conditions.

Appendix I

Suppletory Bayesian Data Analysis

Table I1.

Statistical output for a Bayesian mixed ANOVA of reaction time (ms).

Effect	P(incl)	P(incl | data)	$BF_{Inclusion}$
Time	0.114	0.486	665020.307
Cue	0.114	0.002	9.950e +93
Flanker	0.114	0.001	3.686e +143
Condition	0.114	0.336	0.646
Time by Cue	0.299	0.103	0.116
Time by Flanker	0.299	0.414	0.714
Time by Condition	0.299	0.056	0.132
Cue by Flanker	0.299	0.993	424.381
Cue by Condition	0.299	0.026	0.057
Flanker by Condition	0.299	0.079	0.198
Time by Cue by Flanker	0.114	0.005	0.120
Time by Cue by Condition	0.114	2.354e -5	0.067
Time by Flanker by Condition	0.114	6.136e -4	0.163
Cue by Flanker by Condition	0.114	4.055e -4	0.078
Time by Cue by Flanker by Condition	0.006	1.439e -10	0.030

Note. P(incl) = prior inclusion probability; P(incl | data) = posterior inclusion probability; $BF_{Inclusion}$ = inclusion Bayes factor.

Table I2.

Statistical output for a Bayesian mixed ANOVA of accuracy (% correct).

Effect	P(incl)	P(incl | data)	$BF_{Inclusion}$
Time	0.114	0.126	0.153
Cue	0.114	4.104e -6	7.998
Flanker	0.114	4.623e -6	2.818e +50
Condition	0.114	0.228	0.337
Time by Cue	0.299	0.023	0.152
Time by Flanker	0.299	0.024	0.159
Time by Condition	0.299	0.009	0.175
Cue by Flanker	0.299	0.998	225955.938
Cue by Condition	0.299	0.055	0.204
Flanker by Condition	0.299	0.04	0.142
Time by Cue by Flanker	0.114	2.765e -4	0.094
Time by Cue by Condition	0.114	2.698e -5	0.129
Time by Flanker by Condition	0.114	3.209e -5	0.202
Cue by Flanker by Condition	0.114	0.002	0.239
Time by Cue by Flanker by Condition	0.006	1.014e -9	0.518

Note. P(incl) = prior inclusion probability; P(incl | data) = posterior inclusion probability; $BF_{Inclusion}$ = inclusion Bayes factor.

Table I3.

Statistical output for a Bayesian mixed ANOVA of N1 amplitude (μV)

Effect	P(incl)	P(incl | data)	$BF_{Inclusion}$
Time	0.114	1.044e -11	4127.11
Cue	0.114	1.026e -9	1.992e +25
Flanker	0.114	1.839e -6	582.706
Condition	0.114	0.24	0.445
Time by Cue	0.299	0.174	9.691e +6
Time by Flanker	0.299	0.175	3955.156
Time by Condition	0.299	0.06	0.151
Cue by Flanker	0.299	0.169	28.018
Cue by Condition	0.299	0.116	0.342
Flanker by Condition	0.299	0.086	0.233
Time by Cue by Flanker	0.114	0.825	4.875
Time by Cue by Condition	0.114	0.003	0.204
Time by Flanker by Condition	0.114	0.003	0.227
Cue by Flanker by Condition	0.114	0.002	0.091
Time by Cue by Flanker by Condition	0.006	3.765e -6	0.492

Note. P(incl) = prior inclusion probability; P(incl | data) = posterior inclusion probability; $BF_{Inclusion}$ = inclusion Bayes factor.

Table I4.

Statistical output for a Bayesian mixed ANOVA of P3 amplitude (μV)

Effect	P(incl)	P(incl | data)	$BF_{Inclusion}$
Time	0.114	0.043	0.19
Cue	0.114	0.22	5.731e +46
Flanker	0.114	0.654	4.095
Condition	0.114	0.288	0.634
Time by Cue	0.299	0.718	13.534
Time by Flanker	0.299	0.086	0.15
Time by Condition	0.299	0.058	0.158
Cue by Flanker	0.299	0.053	0.067
Cue by Condition	0.299	0.182	0.502
Flanker by Condition	0.299	0.062	0.153
Time by Cue by Flanker	0.114	4.476e -4	0.095
Time by Cue by Condition	0.114	0.002	0.094
Time by Flanker by Condition	0.114	2.346e -4	0.308
Cue by Flanker by Condition	0.114	1.287e -4	0.095
Time by Cue by Flanker by Condition	0.006	3.917e -10	0.101

Note. P(incl) = prior inclusion probability; P(incl | data) = posterior inclusion probability; $BF_{Inclusion}$ = inclusion Bayes factor.